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EVALUATION OF STROMA-FREE HEMOGLOBIN SOLUTIONS AS RESUSCITATIVE FLUIDS FOR THE INJURED SOLDIER

FINAL REPORT

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This study utilized three basic animal models to study the efficacy of Stroma-free Hemoglobin solutions (SFHS). The models, basically designed to evaluate cardiovascular physiology, consisted of: 1) a swine right heart bypass model detailed for myocardial evaluation, 2) a minimally instrumented exercising canine preparation to evaluate the general ability of SFHSs to support stress, and 3) an extensively instrumented exercising swine model designed to evaluate detailed hemodynamic function during stress. These studies all demonstrated the general efficacy of SFHS even with the 50% exchange used here. The SFHS was more efficacious in preserving normal physiology both at rest and exercise than was a non-oxygen carrying solution (7% albumin) used for comparison. It was not possible to demonstrate a definite benefit when a SFHS modified to improved oxygen off-loading was used and compared with an unmodified SFHS having a relatively low P-50 (approximately 15 Torr).

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SUMMARY

This contract, which ran for two years from September 15, 1979 through October 31, 1981, was designed to evaluate the effectiveness of current hemoglobin solutions as they could be applied for the resuscitation of combat injured soldiers. The contract comprised essentially three studies: (1) evaluation of two different hemoglobin solutions using an acute right heart bypass swine model to carefully evaluate myocardial function, (2) evaluation of two different solutions using a minimally instrumented canine model to determine the general utility of the solutions in an awake, exercising model, and (3) evaluation of the current non-modified hemoglobin solution using an exercising swine model with more extensive instrumentation.

The first study used an amplified animal model previously developed by the author, the other two studies used chronic animal models developed for the purpose of this contract in consultation with members of UCSD. The evaluations utilized measurements of blood gases, oxygen contents, blood lactate, cardiac and peripheral pressures, total body and organ blood flows(directly and with radio-labeled microspheres), cardiac dimensions(utilizing sonomicrometry), and exercise performance.

Results from the first study, which evaluated the basic hemoglobin solution without modification and a modified solution with a higher P-50. Sonomicrometry was used to measure myocardial dimensions, but even with this improved methodology it was not possible to demonstrate a significant improvement in cardiac function with the hemoglobin solution over an albumin solution at the 50% exchange level. The two hemoglobin solution groups had significantly higher arterial contents, and higher mean myocardial oxygen consumption and arterial-coronary sinus oxygen differences, but these differences did not result in any improved cardiac function on the part of the hemoglobin animals. Animals receiving the modified hemoglobin solution did have a slightly higher in-vivo P-50, but no other physiologic changes could be determined.

The exercising canine study again looked at both hemoglobin solutions and compared them with a similar 50% albumin exchange. The animals were minimally instrumented to allow for blood sampling and heart rate monitoring during exercise. Although no major advantage could be discerned from the modified solution over the unmodified solution, animals that received either of these solutions were able to exercise significantly more than the albumin exchanged animals. Furthermore these same hemoglobin solution animals had lower resting and recovery heart rates. As was the case in study #1 the hemoglobin solution animals had higher arterial oxygen contents than the albumin transfused animals.

The third and final study in this contract used a heavier instrumentation in a swine model and compared unmodified hemoglobin solution with a 50% albumin exchange. In addition to the more complex hemodynamic instrumentation, these animals also had microsphere determinations of organ flow during rest and exercise. Mean exercise performance was better with the hemoglobin solution, although not significantly so as was the case with the canine study. Abnormalities in blood flow distribution were generally noted with the albumin solution, whereas the hemoglobin animals had blood flow responses that were essentially unchanged from the initial non-exchanged condition. Exercise arterial-venous oxygen content differences and arterial lactates were better with the hemoglobin solution. Myocardial mechanics, however were not effected by the hemoglobin solution, and the albumin exchanged animals had similar indices of myocardial performance.

Our basic conclusion from these three studies is that even a basic unmodified solution is effective and non-toxic in providing an increased oxygen content that is then translated into improved hemodynamic performance. The major problems of improving oxygen off loading and increasing intravascular retention persist, however we feel that these results provide encouragement in the development of a hemoglobin solution that is an effective blood substitute.

FOREWORD

This report contains no copyrighted material, and there is no material designated for limited distribution. Citations of commercial organizations and trade names in this report do not constitute an official Department of the Army endorsement or approval of the products or services of these organizations.

In conducting the research described in this report, the investigator adhered to the "Guide for the Care and Use of Laboratory Animals," prepared by the committee on Care and Use of Laboratory Animals of the Institute of Laboratory Animal Resources, National Research Council (DHEW Publication No. (NIH) 78-23, Revised 1978).

BODY OF REPORT

STUDY #1

This initial study which was designed to examine the response of the myocardium to a rapid exchange transfusion with either a hemoglobin solution or an albumin solution utilized a perfused swine right heart by-pass model. This model represented an extension of a similar model developed by the principal investigator at Letterman Army Institute of Research prior to the initiation of this contract. The model itself underwent significant development during the time of this contract that consisted primarily of the addition of ultrasonic crystal measurements of myocardial dimensions. Standard, previously developed transducers were applied to this model. The addition of this new technology has allowed us to make more accurate determinations of changes in contractility, as well as the determinations of general hemodynamics and metabolic responses previously available in the older model. The sonomicrometry technology continues to be developed and refined so that consistent and accurate measurements can be obtained. The major technical problems relating to the application of this technology to the swine model were essentially solved during the contract period however.

The results from this initial study have been published(1,2). Reprints of these publications are available in the appendix, and only a general summary and conclusions are presented here. This study basically involved performing an exchange of approximately 50% of the animal's blood volume while the animal was on cardiopulmonary bypass. This resulted in a reduction of the animals normal hematocrit of 30% down to 15%. Exchange transfusions were accomplished using approximately 2 liters of either a 7% albumin solution, a standard unmodified stroma free hemoglobin solution(SFHS), or a stroma free hemoglobin solution modified to increase its P-50 from 15 to 25 Torr. The transfusion of either the unmodified or modified solution at the 50% level resulted in only a modest difference in the "in-vivo" P-50 of the transfused animal that did not reach statistical significance. This lack of a significant difference in the "in-vivo" P-50 of the two SFHS groups was probably responsible for our failure to find any significant differences between the cardiac performance or metabolism in these groups.

We used stroke volume at 14 Torr LVEDP and percent segmental shortening as our primary measurements of left ventricular function. Myocardial performance did decrease significantly in all groups, and although this decrease was largest in the albumin exchanged group, there was no statistically significant difference between the decreases in the three groups. Coronary blood flow, measured by direct drainage in this study, increased in all groups. This increase, which was most probably due to the anemia sustained by all animals, was not significantly different between the three groups. As expected, arterial oxygen content was significantly decreased in all groups, and this decrease was significantly greater in the albumin group. The decreased arterial oxygen content was apparently not below the critical value in any group however, since there were no significant differences in myocardial oxygen consumption, arterial coronary sinus oxygen content difference, or coronary sinus $P0_2$ either within or between groups. Lactate measurements were obtained to determine if the post transfusion anemia would result in any significant anaerobic metabolism. Again our level of anemia was probably above the critical value, and no group manifested lactate production, or a significant drop in lactate production.

The arterial oxygen content data supports the concept that transfusion with stroma-free hemoglobin solution in a 50% exchange model does result in more oxygen availability for the myocardium than if a non-oxygen carrying solution is used. Although trends in myocardial performance also suggest an advantage with SFHS, the magnitude of that advantage us surprisingly small. The reduced arterial oxygen content with albumin in this 50% exchange did not result in a marked reduction in myocardial performance that was significantly reversed by the use of SFHS. Also of interest was the finding that the increased arterial content with either SFHS did not result in any advantage in terms of coronary blood flow, myocardial oxygen consumption, arterial coronary sinus oxygen content difference, coronary sinus PO_2, and lactate extraction.

STUDY #2

The second study represented our first attempt to evaluate the effects of SFHS exchange transfusion in awake animals not subject to the effects of anesthesia and the surgical stress of cardiopulmonary bypass. Minimally instrumented dogs were used in this study which evaluated hemodynamic performance both awake and with exercise. This study again attempted to evaluate the three basic solutions evaluated in the first study. The results from this study are partially published in two publications(2, 3), and completely published in a manuscript submitted for publication(4). Copies of these publications are available in the appendix.

Since the details of this study are currently available in published or finished manuscript form, only a brief summary of the results will be presented here. Our major finding in this study consisted of the marked difference in the exercise response of the SFHS exchanged animals versus the albumin exchanged animals. Animals were exercised approximately 30 minutes after their exchange transfusion. The animals receiving either a sham exchange or an exchange with either of the SFHSs were able to exercise at close to their control values, whereas the albumin exchanged animals had a marked decrease in their exercise performance. The minimal instrumentation given to these animals did not allow us to make detailed hemodynamic measurements, but the measured heart rate responses of the animals were markedly different depending on which solution was received. Albumin exchanged dogs had a significantly increased resting heart rate, a lower exercise heart rate, and an elevated recovery heart rate when compared with the other groups. Arterial oxygen content differences, venous PO_2 and arterial-venous oxygen content differences were less in all of the exchanged animals(except for the sham transfused animals), but there were no significant differences between the exchanged groups that would indicate an advantage for the SFHSs. As expected the imposition of an exercise stress resulted in elevated lactate values in all groups. The albumin animals had an elevated mean resting lactate after exchange that suggests some anaerobic metabolism in these animals even at rest.

The results from this study uniquely show that there is a difference in the exercise response of the animals depending on whether a SFHS, or or an albumin solution is used. This difference was apparent even though the exchange was only at a 50% level. Of further interest was the fact that when the animals were followed for 7 days their exercise performance returned to normal regardless of the solution that was used for the animal's exchange.

STUDY #3

The third study combined the more extensive cardiac instrumentation used in the acute swine preparation with the exercise stressed model initiated in our second

study. The instrumentation involved the performance of a thoracotomy and the subsequent placement of various sampling catheters as well as the placement of a high fidelity pressure transducer. The operated animals, which had previously been trained to run on a treadmill, were allowed to recover from their operation prior to being tested with and without exercise before and after an exchange transfusion. Since our previous two studies had failed to show any significant effect of an improved P-50, we did not evaluate all three solutions, but merely concentrated on the unmodified SFHS and the albumin solution. The details of this study are partially presented in three publications(2,3,6), and in a finished manuscript currently submitted for publication(5). These detailed documents are available in the appendix and therefore a summary of our results is presented below. The results are probably best presented by looking at the changes that occurred with rest and exercise in the albumin exchanged animals, and in the SFHS animals.

Exchanging the animals with albumin solution even at rest resulted in a decreased arterial oxygen content and arterial venous oxygen content difference as well as an increase coronary blood flow and cardiac output. Largely as a result of this increased cardiac output total body oxygen consumption did not change. Myocardial function as measured primarily by sonomicrometrically determined dimension changes was also unchanged. With the imposition of an exercise stress there were additional marked differences in oxygen consumption, total oxygen transport, and aortic pressure. Lactate production and left arterial pressure increased, and there were changes in organ flow that consisted primarily of increases in coronary and cerebral flow, and decreases in visceral organ flow. These albumin exchanged animals also had a reduction in their exercise performance that was slightly greater than the SFHS animals described below.

The SFHS exchanged animals incurred a significant drop in their arterial oxygen content, but otherwise had values similar to those obtained prior to exchange transfusion. These SFHS exchanged animals did demonstrate a drop in their arterial-venous oxygen content difference with exercise, but unlike the albumin animals these SFHS animals showed no changes in oxygen consumption, oxygen transport, lactate production, heart rate, dF/dt, or organ flood flow.

The findings from this study appear to support the importance of having some oxygen carrying capacity in the exchange transfusion solution, even if the level of exchange is only 50%. The effects of the non-oxygen carrying albumin solution were seen primarily in the form of a hyperdynamic performance seen in the resting condition. The animals exchanged with SFHS did not increase their myocardial performance until exercised. It would appear from this study that increased cardiac performance is required to support the metabolic needs of the body if oxygen carrying capacity is not included in the exchange solution. Following an exchange transfusion with the SFHS the animals had rest and exercise responses that were consistently indistinguishable from the control values. These hemodynamic results, as well as the microsphere determined blood flow measurements suggest that SFHS may have a significant value in normalizing the animals cardiovascular response, even if the exchange is only for 50% of the animal's blood volume.

GENERAL CONCLUSIONS

There are certain conclusions that seem to be supported in part by all of the studies summarized here. These studies document the general efficacy of even a basic SFHS without modifications to enhance either its oxygen off-loading ability or its

intravascular retention. This efficacy was not demonstrated in every situation, but could be generally appreciated even when the exchange transfusion resulted in only a 50% reduction in the hematocrit. It would also appear that the solutions as tested in our animal models are relatively non-toxic, as no animal had an adverse reaction to the SFHS exchanges. The issue of the importance of P-50 and improving the oxygen off loading characteristics of the solution would appear to be insufficiently evaluated with these studies. Although we were able to consistently produce a SFHS that had a significantly increased P-50, in no case were we able to demonstrate that the "in-vitro" P-50 had a sufficient "in-vivo" effect when evaluated in our 50% exchange models.

The results from this series of studies offers encouragement regarding the use of SFHS as a usable blood substitute. It would seem appropriate to follow these studies with additional studies evaluating current improved solutions, utilized at various levels of exchange.

LITERATURE CITED

1. Moores WY, FC White, C Bloor, AG Greenburg, R Mack, DC Willford. The Physiologic Effects of Oxygen Transport by Hemoglobin Solutions. In Advances in Blood Substitute Research. Alan R. Liss, Inc., New York, 1983. p 89-99.

2. Moores WY, Sansonetti D, Greenburg AG, Mack RE, Willford DC, Scheussler R. Hemodilution in cardiopulmonary bypass: Efficacy of stroma free hemoglobin solution as a hemodiluting prime during cardiopulmonary bypass. In Thirty Years of Extracorporeal Circulation. Edited by S. Hagl, et al. Carl Gerber, Munich, Germany, 1985.

3. Moores WY: Hemodynamic efficacy of stroma-free hemoglobin solutions as demonstrated in multiple animal models. La Transfusione del Sangue 33:88-103, 1988.

4. Mack RE, WY Moores, FC White, B Guth, DC Willford, AG Greenburg, CM Bloor. Improved Exercise Performance in Hemodiluted Pigs with Stroma-Free Hemoglobin Solution. (Manuscript submitted for publication to J. Appl. Physiol. 1988)

5. Moores WY, K Gallagher, D Sansonetti, RE Mack, J Lindsey, R Schuessler, S Kemper Chronic Exercise Response of the Dog Following Hemodilution induced with Albumin and Stroma-Free Hemoglobin Solutions. (Manuscript submitted for publication to Surg. Gyn. and Obst. 1988).

6. Moores WY, Mack RE, White FC, Bloor CM: Coronary flow dynamics in swine following partial exchange transfusions with hemoglobin and albumin solutions. In Blood Substitutes. Eds: Chang TMS, Geyer RP. Marcel Dekker, Inc. New York, 1989.

CHRONOLOGICAL BIBLIOGRAPHY OF PUBLICATIONS SUPPORTED BY THE CONTRACT

1. Moores WY, FC White, C Bloor, AG Greenburg, R Mack, DC Willford. The Physiologic Effects of Oxygen Transport by Hemoglobin Solutions. In Advances in Blood Substitute Research. Alan R. Liss, Inc., New York, 1983 p 89-99

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6. Moores WY, Mack RE, White FC, Bloor CM: Coronary flow dynamics in swine following partial exchange transfusions with hemoglobin and albumin solutions. In Blood Substitutes. Eds: Chang TMS, Geyer RP. Marcel Dekker, Inc. New York, 1989.

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APPENDIX

1. Moores WY, FC White, C Bloor, AG Greenburg, R Mack, DC Willford The Physiologic Effects of Oxygen Transport by Hemoglobin Solutions In Advances in Blood Substitute Research. Alan R. Liss, Inc., New York, 1983. p 89-99.

2. Moores WY, Sansonetti D, Greenburg AG, Mack RE, Willford DC, Scheussler R: Hemodilution in cardiopulmonary bypass: Efficacy of stroma free hemoglobin solution as a hemodiluting prime during cardiopulmonary bypass. In Thirty Years of Extracorporeal Circulation. Edited by S. Hagl, et al. Carl Gerber, Munich, Germany. 1985

3. Moores WY: Hemodynamic efficacy of stroma-free hemoglobin solutions as demonstrated in multiple animal models. La Transfusione del Sangue 33:88-103, 1988.

4. Mack RE, WY Moores, FC White, B Guth, DC Willford, AG Greenburg, CM Bloor. Improved Exercise Performance in Hemodiluted Pigs with Stroma-Free Hemoglobin Solution. (Manuscript submitted for publication to J. Appl. Physiol. 1989)

5. Moores WY, K Gallagher, D Sansonetti, RE Mack, J Lindsey, R Schuessler, S Kemper.Chronic Exercise Response of the Dog Following Hemodilution induced with Albumin and Stroma-Free Hemoglobin Solutions. (Manuscript submitted for publication to Surg. Gyn. and Obst. 1989)

6. Moores WY, Mack RE, White FC, Bloor CM: Coronary flow dynamics in swine following partial exchange transfusions with hemoglobin and albumin solutions. In Blood Substitutes. Eds: Chang TMS, Geyer RP, Marcel Dekker, Inc. New York , 1989

(2)

W. Y. Moores, D. Sansonetti, A. G. Greenburg, R. E. Mack, D. Willford, R. Schuessler

Hemodilution on cardiopulmonary bypass: Efficacy of stroma free hemoglobin solution as a hemodilution prime during cardiopulmonary bypass

Introduction

Induced hemodilution is essentially universally accepted as appropriate for the conduct of almost all cardiac procedures utilizing cardiopulmonary bypass. In addition to the obvious benefit of decreasing the utilization of homologous blood transfusions, induced hemodilution has value in improving peripheral circulation by virtue of the decreased viscosity afforded by a lower hematocrit. This decreased viscosity is especially advantageous if hypothermic cardiopulmonary bypass is utilized. The major detriment to extending the hemodilution to an extremely low hematocrit is that profound hemodilution is accompanied by a marked reduction in blood oxygen capacity. Current hemodiluting solutions such as fluorocarbon emulsions and stroma free hemoglobin solutions have theoretical advantages in this respect since they can carry significantly greater amounts of oxygen than that present in dissolved form in plasma. As attractive as these solutions appear, there remains significant concern regarding the actual amount of oxygen carried by these solutions, the physiologic importance of the increased oxygen capacity, and the toxicity of the solutions.

Crude hemoglobin solutions have a history dating back to 1935 (1), while a usable stroma free hemoglobin solution without kidney damaging characteristics was introduced by Rabinet in 1967 (2). Fluorocarbon solutions have likewise had a history of close to 20 years with the initial biological utilization in 1966 by Clark and Gollen (3). Almost all of the studies evaluating these compounds have been in subtotally exchanged animals where there was little or no native blood to interact with the solutions. Furthermore, only a few studies having examined the utilization of these solutions as cardiopulmonary bypass primes with the primary objective of evaluating myocardial metabolism and function.

Funding for this study provided through the Veterans Administration and the United States Army, Research and Development Command.

The intent of this study was to evaluate the effectiveness and safety of stroma free hemoglobin solutions at a moderate level of hemodilution in a cardiopulmonary bypass environment. Since oxygen offloading is of concern with unmodified stroma free hemoglobin solution having a low P-50, we examined a solution chemically modified to improve oxygen offloading, as well as a standard unmodified solution. We wish to specifically test the hypothesis that stroma free hemoglobin solution is a safe cardiopulmonary bypass prime solution and that it can provide improved myocardial physiologic performance due to the increased availability of oxygen. Furthermore, we postulated that the improved oxygen offloading characteristics of the modified solution would also have a measurable beneficial physiologic effect.

Methods

A. Surgical preparation

15 immature swine of either sex weighing approximately 20 kg were utilized for this study. All animals were anesthetized and surgically prepared as previously described by us (4) with the modification that 2 pairs of sonomicrometers were placed to measure myocardial segment dimension changes. All animals were induced with oxygen and halothane (2% or less) and endotracheal access was obtained to support ventilation with a Harvard animal volume respirator. Catheters were placed in the femoral vein for administration of I.V. fluids and medications while an additional catheter was placed in the carotid artery for pulse and pressure monitoring. The halothane was discontinued after 10 minutes or less and anesthesia was maintained with morphine sulphate while the animals were paralyzed with a continuous succinyl choline I.V. drip. Morphine sulfate was given in 15 mg injections in sufficient quantity to keep the systolic blood pressure less than 120 torr and the heart rate less than 130 beats per minute. Following institution of adequate anesthesia, a median sternotomy was performed and the animals were cannulated for right and total cardiopulmonary bypass as illustrated in Figure 1. The atrial-ventricular conduction system was blocked with an intraseptal injection of formalin to facilitate subsequent constant ventricular pacing at a constant rate of 140. An aortic tourniquet was utilized to increase aortic root pressure during low flows, while an arterial venous shunt in the perfusion circuit was used to decrease root pressure during high flows. Two sets of myocardial segment ultrasonic crystals were placed in the mid and apical anterior left ventricular wall to measure segmental motion. The crystals were oriented in a circumferential plane. These crystal transducers were connected to an ultrasonic dimension system (Schuesser and Assoc., Cardiff By The Sea, CA). Catheter pressures were measured in the carotid artery and left ventricle, while a high fidelity left ventricular pressure signal was obtained with a Konnigsberg transducer. All pressure and dimension signal outputs were continuously recorded on FM magnetic tape and a Gould physiologic recorder.

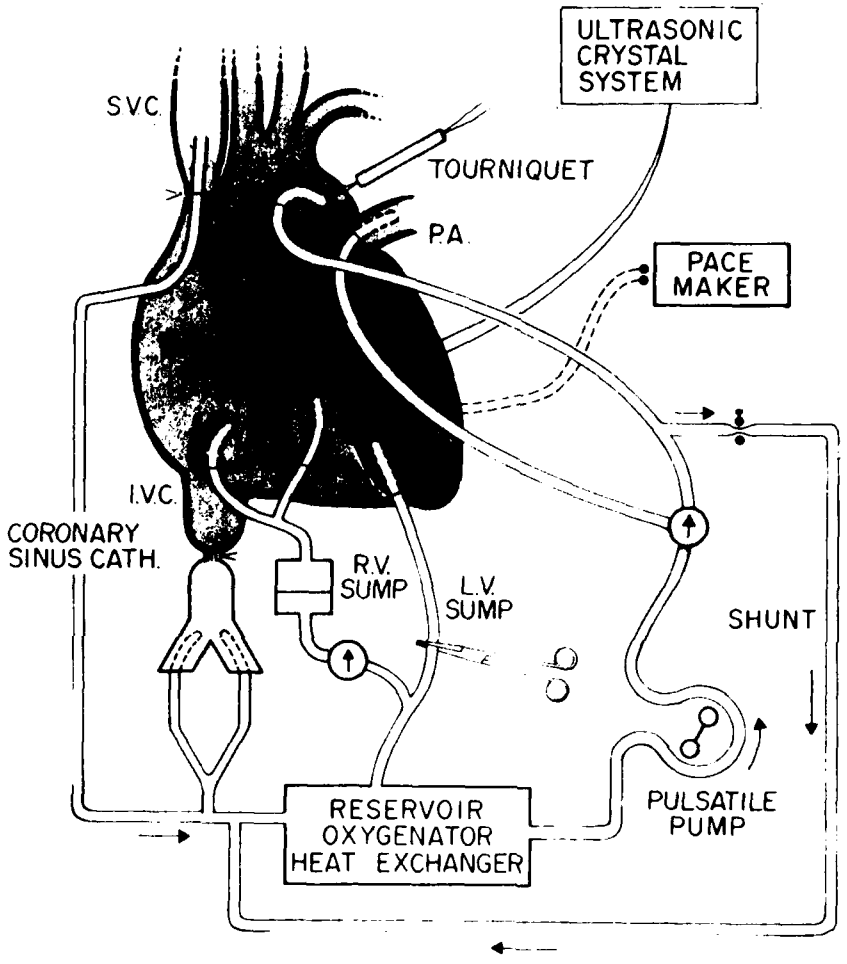

Figure 1. *Diagram of right heart bypass circuit. SVC, superior vena cava. IVC, inferior vena cava. LA, left atrium. RA, right atrium. LV, left ventricle. PA, pulmonary artery.*

Following cannulation and instrumentation, the animals were placed on total cardiopulmonary bypass utilizing a Sarns perfusion pump. Myocardial function was evaluated by switching flow from the aorta to the pulmonary artery and facilitating right heart bypass with the left ventricle performing both pressure and volume work. With the mean arterial blood pressure kept as close to 80 torr as possible, left ventricular function curves were constructed by changing pump flow to provide a variation in left ventricular end diastolic pressure from 6 to 14 torr. For comparison purposes stroke volume is presented as that pump flow divided by 140 that resulted in a measured left ventricular end diastolic pressure of 12

torr. Percent myocardial segment shortening was determined by taking the millimeters of shortening and dividing by the segment length during diastole. Coronary blood flow was determined by measuring the collected blood from the coronary sinus and right ventricle over 30 seconds. Standard blood gases were determined with an Instrumentation Laboratories 213 blood gas analyzer and an Instrumentation Laboratories co-oximeter. Myocardial oxygen consumption was represented by multiplying the coronary blood flow times the arterial coronary sinus oxygen content difference and was expressed as milliliters of oxygen per 100 grams of heart tissue. Lactate determinations were taken in both arterial and coronary sinus blood and lactate extraction was calculated as the difference between these two determinations divided by the arterial lactate value. The actual lactate determinations were done according to standard laboratory techniques. P-50 determinations (blood PO_2 at 50% hemoglobin saturation) were calculated using all PO_2 and oxygen saturation values obtained during a control or post exchange dilution condition.

B. Experimental sequence and animal group

All animals were subjected to baseline control measurements at a pre exchanged hematocrit of 30%. A rapid exchange transfusion was subsequently carried out utilizing approximately 2 liters of solution to effect a post-exchange hematocrit of approximately 15%. All measurements were repeated and represented the dilution values.

The 15 animals were divided into three equal groups with each group receiving one of the three following solutions:

1. 7% bovine serum albumin
2. Stroma free hemoglobin solution prepared by filtration and centrifugation, and
3. Similarly prepared stroma free hemoglobin solution modified with pentidoxal phosphate to decrease its oxygen affinity (5).

The characteristics of a typical batch of both the unmodified and modified stroma free hemoglobin solutions are given in Table 1.

	Unmodified Hemoglobin	Modified Hemoglobin
Total Hemoglobin	6.1 gm/DL	6.4 gm/DL
% Methemoglobin	0.9 %	2.2 %
P–50	14.9 Torr	23.3 Torr
Osmolarity	312 m/Osm	311 m/Osm
Sodium	138 mE/L	142 mE/L
Potassium	8 mE/L	4.7 mE/L

Table 1: *Characteristics of hemoglobin solutions*

C. Statistical Analysis

Statistical comparison within groups were made using paired t-test analysis. To evaluate differences between groups, one way analysis variance was used. A statistical significance difference was felt to be achieved when the p value was less than 0.05.

Results

All results are presented in Tables 2 and 3. The values for percent shortening, arterial oxygen consumption and myocardial oxygen consumption are further represented in histograms. (Figures 2, 3, and 4).

	Albumin	
	Control	Dilution
Hematocrit (%)	28±5	12±1*
P-50 (Torr)	35±2	33±4*
Stroke Volume (ML)	20±9	10±2*
Shortening (%)	12±5	9±2*
	Unmodified Hemoglobin	
	Control	Dilution
Hematocrit (%)	26±4	13±2*
P-50 (Torr)	34±1	26±3*
Stroke Volume (ML)	21±5	15±4*
Shortening (%)	13±2	10±16*
	Modified Hemoglobin	
	Control	Dilution
Hematocrit (%)	26±2	12±1*
P-50 (Torr)	37±1	28±2*
Stroke Volume (ML)	16±4	12±4*
Shortening (%)	13±2	9±3*

* $P < 0.05$ Control versus Dilution.
* $P < 0.05$ Albumin Dilution versus other Dilution.

Table 2: *Hemodynamic measurements before and after hemodilution. P-50, PO_2 at 50% saturation. * $P < 0.05$, paired t test, control versus dilution. + $P < 0.05$, one way analysis of variance, albumin dilution versus other dilutions.*

Hemodynamics

Table 2 summarizes values for hematocrit, P-50, stroke volume and percent shortenings before and after hemodilution. As can be seen from the hematocrit values we achieved an approximate 50% exchange transfusion in each group. P-50 values dropped significantly in both hemoglobin solution groups as a result of hemodilution with hemoglobin having a lowered P-50. The post dilution P-50 values for the albumin group were unchanged from control and significantly higher than the post dilution values with both hemoglobin solutions since the albumin group did not have any dilution of the native hemoglobin with hemoglobin having differing P50 values. Although the P50 of the diluting modified hemoglobin solution was greater than the unmodified solution (24 torr vs 15 torr), this difference was not statistically discernable following the 50% in vivo exchange.

Myocardial function as characterized by stroke volume and percent shortening revealed a statistically significant decrease in every group and no differences between groups could be demonstrated.

Coronary blood flow was noted to increase in all three groups following exchange, but the wide variability precluded statistical verification of this increase. Even if the wide variation were ignored and mean value comparisons were made between groups, there was no noticable effect on utilizing hemoglobin solution as the diluting solution when compared to utilization of albumin solution.

	Albumin		Unmodified Hemoglobin		Modified Hemoglobin	
	Control	Dilution	Control	Dilution	Control	Dilution
Arterial Oxygen Content (Ml/DL)	11±2	5±1*	11±1	8±1*	11±1	9±1*
Myocardial Oxygen Consumption (Ml/min/100 g)	9±5	3±1	7±1	6±4	6±1	7±3
Arterial-Coronary Sinus Oxygen Difference (Ml/DL)	5±2	2±1	3±1	2±1	3±1	2±1
Coronary Sinus PO2 (Torr)	44±11	45±10	52±14	43±12	46±8	41±4
Lactate Extraction %	1±8	2±4	2±4	0±8	1±5	1±2

* $P \leq 0.05$ Control versus Dilution.
† $P < 0.05$ Albumin Dilution versus other Dilution.

Table 3 *Oxygen dynamics before and after hemodilution. * $P < 0.05$, paired t test, control versus dilution. † $P < 0.05$, albumin dilution versus other dilutions.*

Oxygen dynamics

The presence of a higher arterial oxygen content following hemodilution with two hemoglobin solution groups in comparison with the albumin group was the only statistically demonstrated benefit of hemodilution with hemoglobin solutions. Mean myocardial oxygen consumption values suggested that oxygen consumption was better maintained with hemoglobin solutions, but the wide variation again made statistical verification impossible.

Coronary sinus pO_2 and lactate extraction were essentially unchanged following hemodilution with any of the three solutions.

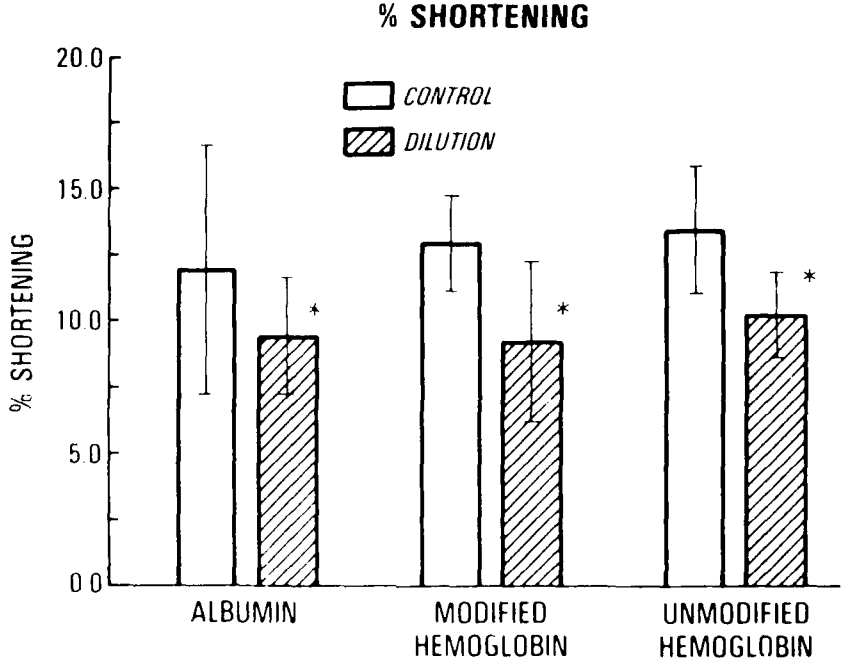

Figure 2 *Histogram showing relatively constant decrease in % shortening following hemodilution with any solution. * $P < 0.05$, paired t test, control vs dilution.*

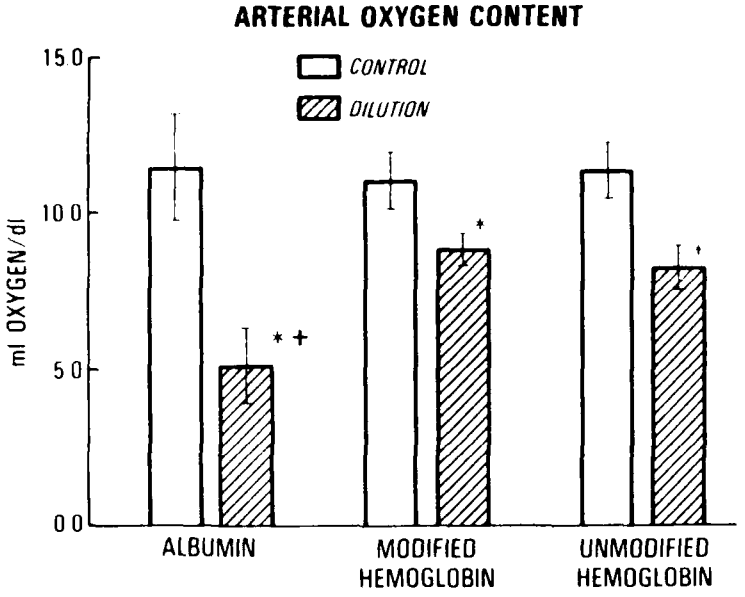

Figure 3: *Histogram demonstrating a significantly greater decrease in oxygen content following hemodilution with albumin solution. * $P < 0.05$, paired t test, control vs. hemodilution, + $P < 0.05$ one way analysis of variants, albumin versus others.*

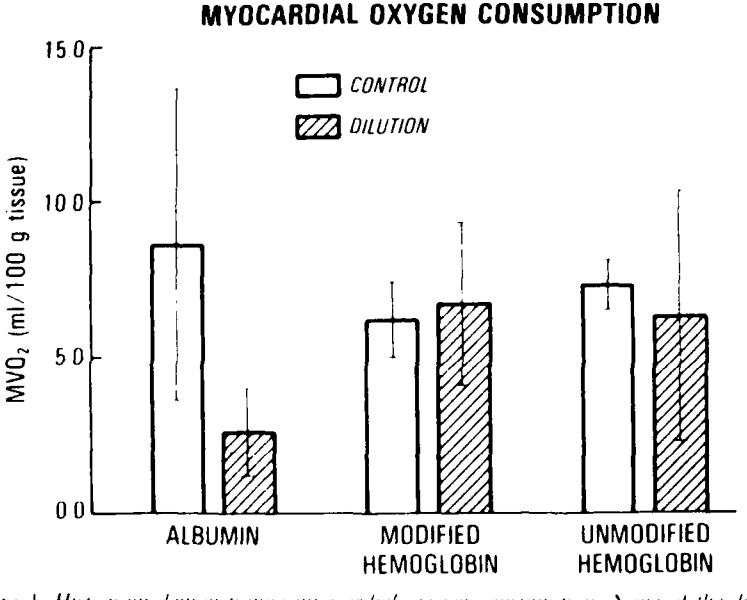

Figure 4. *Histogram demonstrating myocardial oxygen consumption. None of the differences reached statistical significance.*

Discussion

In performing hemodilution on cardiopulmonary bypass at a 50% exchange level, we noted generally satisfactory maintenance of myocardial function, although all groups were noted to have statistically verifiable decreases in stroke volume. Previous experience with this animal model indicated generally well preserved myocardial function if the hematocrit was kept at 15% or greater (6). That same report documented a loss of myocardial function if the hematocrit was at 10% or less. The increased oxygen capacity provided by the hemoglobin was insufficient to prevent the decrease in stroke volume that occurred when the hematocrit reached approximately 12%. *Myocardial contractile function may not be a sensitive* indicator of the benefits of increasing oxygen capacity as other studies examining myocardial function in nonanesthetized animals also failed to show any benefit when *stroma free hemoglobin solution* was used (7,8,9). Given the 50% exchange used in this study, it was not possible to demonstrate any physiologic benefits or effect from utilization of stroma free hemoglobin solution *having improved oxygen offloading characteristics*. When we examined these three solutions in an awake canine model, we were also unable to demonstrate an effect in the immediate post-transfusion period. Although there was a measurable improved exercise performance after the initial 24 hours in those animals given the modified versus the unmodified solution (9). At higher levels of exchange, we have previously demonstrated a physiologic effect when blood of differing P50 characteristics was used (10). However we must conclude that under the conditions of cardiopulmonary bypass, when the degree of exchange is at 50%, there were no demonstrable physiologic benefits from the usage of stroma free hemoglobin solution as opposed to albumin solution. This data does not, however, contradict our earlier study evaluating stroma free hemoglobin solution as a cardiopulmonary bypass prime at extremely low hematocrit levels (11). In that study utilizing a hematocrit of 5%, we were unable to maintain any cardiac function unless the albumin prime was replaced with a stroma free hemoglobin solution prime.

Stroma free hemoglobin solution is not the only oxygen carrying solution being evaluated as a blood substitute and consequently as a cardiopulmonary bypass priming solution. Fluorocarbon emulsions have been evaluated in this role primarily by Engelman (12,13). He noted that these emulsions were generally effective, but also reported some *disturbing increases in pulmonary* vascular resistance, thus raising concern regarding the safety and tolerance of these solutions.

Concern regarding non nephrotic toxicity of the hemoglobin solutions continues to be raised, however we have failed to document any measurable toxicity in our studies exchanging canine and swine models. Stroma free hemoglobin solution is *not* a manufacturable "chemical" solution such as fluorocarbon emulsions, and is subject to preparation variations that may result in toxic sequelae when the solutions are administered to animal models. Obviously, a stroma free hemoglobin solution intended for human use would have to have sufficient quality control and purity to avoid any toxic reaction.

Summary and conclusions

In this study examining three cardiopulmonary bypass priming solutions utilized at the 50% exchange level, we were unable to demonstrate any statistically significant physiologic effects when a stroma free hemoglobin solution was used in preference to albumin solution. We must conclude therefore that the measurable increase in oxygen capacity present in the stroma free hemoglobin solution was not needed at this level of hemodilution on cardiopulmonary bypass. Furthermore, no benefit was achieved by modifying the solution to have improved oxygen offloading characteristics.

Our study does continue to substantiate that those stroma free hemoglobin solutions manufactured and utilized by us in this and other related studies is safe and nontoxic. Furthermore, this study does not contradict our findings that stroma free hemoglobin solution has a significant physiologic benefit if the exchange is carried out to a lower hematocrit, or conducted under non cardiopulmonary bypass conditions.

Abstract

We placed 15 swine on total and right heart bypass and evaluated myocardial function and metabolism before and after a 50% hemodilution with: (1) 7% albumin solution, (2) unmodified stroma free hemoglobin solution, and (3) stroma free hemoglobin solution modified to improve oxygen off-loading. Myocardial function as characterized by stroke volume and sonomicrometry measured segmental shortening decreased significantly ($P < 0.05$) but nondiscriminately with all solutions. A statistically significant ($P < 0.05$) greater arterial oxygen content and a nonstatistically significantly greater myocardial oxygen consumption was present with both stroma free hemoglobin solution dilutions when compared with the albumin solution. Lactate extraction, and coronary blood flow was comparable in all groups. No differences were noted between the two hemoglobin solutions. We conclude that hemodilution on cardiopulmonary bypass with stroma free hemoglobin solution is non-toxic and results in greater oxygen capacity, but this increased capacity is not reflected in improved physiologic performance at a 50% hemodilution.

References

1. Amberson W. R.:
On the use of Ringer-Locke solutions containing hemoglobin as a substitute for normal blood in mammals. J. Cell. Comp. Physiol. 5: 359, 1934

2. Rabiner S. F.:
Evaluation of a stroma free hemoglobin solution for use as a plasma expander. J. Exp. Med. 126: 1127, 1967

3. Clark L. C., Gollen F.:
Survival of mammals breathing organic liquids equilibrated with oxygen at atmospheric pressure. Science 152: 1755, 1966

4. Moores W. Y., Devenuto F., Heydorn W. H., et al:
Extending the limits of hemodilution on cardiopulmonary bypass using stroma free hemoglobin solution. J. Thorac. Cardiovasc. Surg. 81: 155, 1981

5. Greenburg A. G., Schooley M., Peskin G. W.:
Improved retention of stroma free hemoglobin solution by chemical modification. J. Trauma 17: 501, 1977

6. Moores W., Heydorn W., Dembitsky W., Raysinger M., Willford D., Mahoney E.:
Hemodilution and cardiopulmonary bypass: Support of the pig heart at a reduced threshold hematocrit of 15%. Circ 65 (Suppl. II-305), 1982

7. Moores W. Y., White F. C., Bloor C., Greenburg A. G., Mack R., Willford D. C.:
The physiologic effect of oxygen transport by hemoglobin solutions. In Advances in Blood Substitute Research, eds: Bolin, Geyer, Nemo, Alan R. Lists, New York, pg. 89-99, 1983

8. Mack R. B., Moores W. Y., White F. C., Guth B., Willford D. C., Greenburg A. G., Bloor C. M.:
Improved physiologic performance following hemodilution with stroma free hemoglobin solution versus albumin exchange in exercising swine. (Manuscript submitted for publication).

9. Moores W. Y., Gallagher K., Mack R. E., Lindsey J., Schuessler R., Kempler S., Ross Jr. J.:
Chronic exercise response of the dog following hemodilution induced with albumin and stroma free hemoglobin solution (Manuscript submitted for publication)

10. Moores W. Y., Willford D. C., Crum J. D., Neville J. R., Weiskopf R. B., Dembitsky W. P.:
Alteration of myocardial function resulting from changes in hemoglobin oxygen affinity. Circ. 58 (Suppl. II), 225, 1978

11. Moores W. Y., Devenuto F., Heydorn W. H., et al:
Effectiveness of stroma free hemoglobin solution as seen in a right heart bypass swine model. Critical Care Med. 10: 279–282, 1982

12. Engelman R. M., Rousou J. H., Dobbs W. A.:
Fluorsol-DA: An artificial blood for total cardiopulmonary bypass. Ann. Thor. Surg. 32: 528–535, 1981

13. Engelman R. M., Dobbs W. A., Rousou J. H., Anisimowicz L.:
Use of fluorsol-DA during open heart surgery. In: Advances in Blood Substitute Research, eds: Bolin, Geyer, Nemo, Alan R. Liss, New York, p. 273–282, 1983

Discussion Part 3
Pathophysiology of ECC: Organ function (Heart)

Agostoni:
"May I ask the last speaker what is the modified stroma free hemoglobin solution? Which kind of preparation was used?"

Moores:
"In this study, the basic preparation was a solution prepared by filtration and ultracentrifugation. The modification involves wracking the raw hemoglobulin solution with pyridoxal phosphate so that you occupy the 2- 3 DPG site and, therefore, decrease the oxygen affinity. So the modification was primarily the addition of pyridoxal phosphate."

Hagl:
"I have a question for Dr. Buckberg. If I understand your figures correctly, you showed us that subendocardial perfusion during venting is less than under normal conditions. I think you should explain the mechanism. How should this work? Does this mean that it is better to have a distended volume loaded heart instead of a vented heart?"

Buckberg:
"The mechanism of the increase in subendocardial vascular resistance is probably that the subendocardial vessels become distorted when the heart gets small. It is not better to have the heart distended than vented. The point of that slide was to show that potential subendocardial flow is less when you have a small vented heart. This limits reactive hyperemia when you take off the cross-clamp. Certainly, the oxygen demand is less when the heart is vented than when it is distended."

English:
"I also have a question for Dr. Buckberg. I was impressed with his view using multidose cardioplegia, you could preserve the heart effectively for periods up to four hours. I guess what I would like to know is how he translates this into clinical practice. Because there are two aspects to myocardial preservation. There is the aspect of its effectiveness and the aspect of its over-all simplicity and both are of interest to us. So I would be grateful if Mr. Buckberg would just very briefly describe how he would manage a patient who needs four distal coronary grafts with an ordinary ventricle. I would also like Mr. Buckberg's comment on the alternative to myocardial protection which is that that equal protection to multidose cardioplegia can be achieved by a simple single dose to arrest the heart pharmacologically followed by profound topical hypothermia to keep it arrested. We have achieved this in the animal model getting survival after 16 hours of such a preparation."

Buckberg:
"Let me answer your first question first. There is no difference in how we manage patients with normal or abnormal ventricles because we essentially apply the

same techniques to everyone. We give the initial cardioplegia dose for about 3 minutes, using our standard blood cardioplegic solution. We graft the most critical area first, since these areas receive the least distribution of the cardioplegic solution. We then deliver the cardioplegic solution down the graft to continue the operation with the least critical area grafted last. We administer a warm blood cardioplegic reperfusate before removing the aortic clamp (through the grafts and into the aorta). After removal of the aortic clamp, we give warm non-cardioplegic blood through the grafts while we perform the proximal anastomosis. The most critical graft is connected to the aorta last, to provide graft perfusion beyond the stenosis as long as possible. All operations are done exactly the same way and we have obtained satisfactory results using these techniques.
Your second question refers to the strategy of topical cooling, together with single dose cardioplegia. We don't believe this is as effective as our current technique. I do not think the issue is survival, since most patients survive most operations. The critical question of how well the ventricle has been protected has to be answered, not so much in the operating room, but in the months and years post-operatively. We see ventricles which do not contract well in late follow-up when we do not protect them adequately. With single dose cardioplegia, you must realize the cardioplegic solution is gone within 10 minutes because of non-coronary collateral flow, as I pointed out. The heart becomes progressively acidotic, if you measure myocardial pH. Multidose cardioplegia serves several additional purposes. First it restores hypothermia. You will find the heart warms progressively during cardiac operations, since most of the time it must be lifted from the pericardium to perform distal anastomosis. Multidose cardioplegia also restores pH of the myocardium, and most importantly it restores energy stores, especially if you use an oxygenated solution. I do not believe a single dose with topical cooling is an equally acceptable alternative to multidose cardioplegia."

Dr. Bethune:
"As a supplementary question Dr. Buckberg. What is your view on the use of non - blood multiple cardioplegia?"

Buckberg:
"If one is using cardioplegia, one should use multidose because the considerations with non-blood cardioplegia will be essentially the same thing as with blood cardioplegia. Periodic replenishment serves several purposes. It buffers acidosis, washes out metabolites, maintains hypothermia, provides substrate and minimizes edema. Multidose blood cardioplegia provides the added benefit of replenishing high-energy phosphates (CP). These goals cannot be obtained with a single application of cardioplegia. Therefore, I consider multidose important whether you use blood or an asanguineous cardioplegic solution."

Hagl:
"I have got a question for Dr. Moores. I was very surprised to see that in your control group you found a decrease in shortening after hemodilution. We have found the opposite. Due to changes in compliance we observed an increase in shortening due to - what we call - an intrinsic Frank Starling mechanism. Could you comment on that, please?".

Moores:
"I am not quite clear what you mean by control. A hemodilution with albumin might be considered the non-hemoglobin solution and in that case we did find reduction in shortening. If we took the dilution only down to a hematocrit of 15 rather than of 12 which is the case here, we did not get as marked change in systolic function."

Hagl:
"How far did you go down with the hematocrit in your control group?"

Moores:
"In this particular study we went down to a hematocrit of 12. In a previous study we have gone down as low as 5% and in that case there is no function."

Hagl:
"But at a hematocrit of 12%, providing that you have an intact coronary circulation and an intact coronary reserve capacity, you should not have a decrease in local segment shortening. From our studies we would expect an increase in regional myocardial performance."

Moores:
"There may be differences of model. We used a pig. I am not quite sure what your model was but that might possibly explain it. Also, the right-heart bypass preparation is a little bit more severe in terms of taking its toll over time."

Deac
"With multiple dose cardioplegia I think we are not very far from continuous perfusion cardioplegia. I would like Dr. Buckberg to comment on that."

Buckberg:
"We are quite far from continuous perfusion. In a valve operation we give a dose of cardioplegia for 2 minutes every 20 or 30 minutes. In coronary operations, we give doses after each anastomosis is completed. If you measure myocardial temperature, you will find that it is almost impossible to achieve homogeneous cooling by infusion of cardioplegia through the aorta. However, the moment you put cold cardioplegia through a completed graft, that region of the myocardium becomes very cold. This is not continuous perfusion, but an addition to the operation which makes it safer."

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Hemodynamic efficacy of stroma-free hemoglobin solutions as demonstrated in multiple animal models (*)

WILLIAM Y. MOORES

The opinions and assertions contained herein are the private view of the author and are not to be construed as official or as reflecting the views of the Department of the Army or the Department of Defense (AR 360-5).

In conducting the research described in this report, the investigator adhered to the « Guide for the Care and Use of Laboratory Animals », as promulgated by the *committee on revision of* the « Guide for Laboratory Animal Facilities and Care », Institute of Laboratory Animals Resources, National Research Council.

Improvements in obtaining and storing homologous blood for transfusion, as well as increased effiicency in administering blood and blood components has resulted in a current ability to meet most blood replacement needs. Increasing concern for blood-borne viral infections such as hepatitis and AIDS has provided renewed interest for a blood substitute that can be readily available, safe, and efficacious.

Several experimental resuscitation fluids currently have been evaluated. From among these, stroma-free hemoglobin solution appears to have advantages based primarily upon its ability to transport oxygen [1]. In addition, it exists as a naturally occurring protein which can be transfused without any known allergic or cross-matching problems [2]. Stroma-free hemoglobin solution, in the lyophylized state, has a relatively long shelf life and has previously been reported as an « ideal » plasma expander [3]. Also, due to its low viscosity [4], stroma-free hemoglobin solution potentially may be an excellent candidate for initiating hemodilution during cardiopulmonary bypass [5].

Despite these advantages, there are problems associated with the use of stroma-free hemoglobin solutions which need further investigation. One problem concerns the typically low P_{50} value (11-13 *torr*) of hemoglobin solution, resulting in a leftward shift in the oxyhemoglobin dissociation curve with increased oxygen affinity [6]. A second major problem concerns the relatively short biological retention time [11] of hemoglobin solution. In addition, although the kidney-damaging characteristics of the earlier solutions have been resolved with removal of red blood cell stroma [6, 10, 11], recent published reports [12, 13] as well as unpublished communications from various laboratories using stroma-free hemoglobin solutions have raised the question of non-stroma related toxicity in the form of deleterious procoagulant, cardiovascular, and organ damaging effects. These toxic reactions have

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made the solution virtually unusable for many of these investigators. Initial investigations into the cause of these toxic reactions indicate that some animal models (e.g., rabbit) may be inappropriate, and that special care must be exercised to insure a high level of consistent quality control in the production of the solution as well as insuring that the final solution is chemically balanced and free of endotoxin and pyrogens. Since we did not experience any of these non-stromal associated problems with our particular solutions, we have not addressed this toxicity issue in our studies, but have concentrated primarily on evaluating the effects of high oxygen hemoglobin affinity and shortened retention time in solutions used to effect hemodilution in various exchange animal models.

Two of these problems of concern in our studies have been addressed by Greenburg and associates who have reported that pyridoxalation of stroma-free hemoglobin solution with pyridoxal 5-phosphate (PLP) not only improved P_{50} values without impairing oxygen carrying capacity, but also improved intravascular retention time over unmodified versions by 50%. More recent work from Europe by Kothe and associates has resulted in further solution improvements utilizing intermolecular crosslinking as well as pyridoxalization.

Our studies were designed to compare the effects of three different hemodiluting fluids: 1) modified stroma-free hemoglobin solution, 2) unmodified stroma-free hemoglobin solution, and 3) 7% albumin solution in both dogs and pigs exchanged to equal hematocrit levels and examined under various physiologic conditions. We specifically wished to determine if the greater oxygen-carrying capacity of either stroma-free hemoglobin solution provides any significant advantage in terms of supporting myocardial function and total body hemodynamics (at a reduced circulating hematocrit).

This report summarizes our experience utilizing three different animal models to evaluate the efficacy of stroma-free hemoglobin solutions under different physiologic situations. We used: 1) a right heart bypass preparation in swine to make detailed evaluations of myocardial function and metabolism; 2) an awake, exercising dog model, minimally instrumented without a thoracotomy, to evaluate the general hemodynamic response to stroma-free hemoglobin solution hemodilution; and 3) a more extensively instrumented exercising swine model designed to evaluate post-exchange hemodynamics in more detail.

Swine Right Heart Bypass Studies

Methods

Surgical preparation

Fifteen immature swine of either sex weighing approximately 20 Kg were utilized for this study. All animals were anesthetized and surgically prepared as previously described by us[1] with the modification that two pairs of sonomicrometers were placed to measure myocardial segment dimension changes. All animals were induced with oxygen and halothane (2% or less) and endotracheal access was obtained to support ventilation with a Harvard animal volume respirator. Catheters were placed in the femoral vein for administration of I.V. fluids and medications while an additional catheter was placed in the carotid artery for pulse and pressure monitoring. The halothane was discontinued after ten minutes or less and anesthesia was maintained with morphine sulphate while the animals were paralyzed with a continuous succinyl choline I.V. drip. Morphine sulfate was given in 15 mg injections in sufficient quantity to keep the systolic blood pressure less than 120 torr and the heart rate less than 130 beats per minute. Following institution of adequate anesthesia, a median sternotomy was performed and the animals were cannulated for right and total cardiopulmonary bypass. The atrial-ventricular conduction system was blocked with an intraseptal injection of formalin to facilitate subsequent constant ventricular pacing at a constant rate of 140. An aortic tourniquet was utilized to increase aortic root pressure during low flows, while an arterial venous shunt in the perfusion circuit was used to decrease root pressure during high flows. Two sets of myocardial segment ultrasonic crystals were placed in the mid and apical anterior left ventricular wall to measure segmental motion. The crystals were oriented in

a circumferential place. These crystal transducers were connected to a dimension system (Schuessler and Assoc., Cardiff by the Sea, CA [S.A.]). Catheter pressures were measured in the carotid artery and left ventricle while a high fidelity left ventricular pressure signal was obtained with a Konnigsberg transducer. All pressure and dimension signal outputs were recorded on a Gould physiologic recorder.

Following cannulation and instrumentation the animals were placed on total cardiopulmonary bypass utilizing a Sarns perfusion pump. Myocardial function was evaluated by switching flow from the aorta to the pulmonary artery and facilitating right heart bypass with the left ventricle performing both pressure and volume work. With the mean arterial blood pressure kept as close to 80 ± 5 mm as possible, left ventricular function curves were constructed by changing pump flow to provide a variation in left ventricular end diastolic pressure from 0 to 14 mm Hg. For comparison purposes stroke volume is presented as that pump flow divided by 14 that resulted in a measured left ventricular end diastolic pressure of 12 mm Hg. Percent myocardial segment shortening was determined by taking the difference of shortening and dividing by the segment length during diastole. Coronary blood flow was determined by measuring the collected blood from the coronary sinus and right ventricle over 30 seconds. Standard blood gases were determined and myocardial oxygen consumption was represented by multiplying the coronary blood flow times the arterial coronary sinus oxygen content difference. Lactate determinations were taken in both arterial and coronary sinus blood and lactate extraction was calculated as the difference between these two determinations divided by the arterial lactate value. P_{50} determinations (blood PO_2 at 50% hemoglobin saturation) were calculated using all PO_2 and oxygen saturation values obtained during a control or post-exchange dilution condition.

Experimental sequence and animal group

All animals were subjected to baseline control measurements at a pre-exchange hematocrit of 30%. A rapid exchange transfusion was subsequently carried out utilizing approximately 15%. All measurements were repeated and represented the dilution values.

The 15 animals were divided into three equal groups with each group receiving one of the three following solutions: 1) 7% bovine serum albumin; 2) stroma-free hemoglobin solution prepared by filtration and centrifugation; and 3) similarly prepared stroma-free hemoglobin solution modified with peridoxal phosphate to decrease its oxygen affinity[35].

Statistical analysis

Statistical comparison within groups were made using paired *t*-test analysis. Two evaluate differences between groups one-way analysis variance was used. A statistically significant difference was felt to be achieved when the P value was less than 0.05.

Results

All results are presented in tables I and II.

Hemodynamics

Table II summarizes value for hematocrit, P_{50}, stroke volume and percent short-

Table I

Hemodynamic measurements before and after hemodilution
P_{50} = PO_2 at 50% saturation

	Albumin		Unmodified hemoglobin		Modified hemoglobin	
	Control	Dilution	Control	Dilution	Control	Dilution
Hematocrit	29.5	12.1	26.4	13.2	29.2	12.1
P_{50}	35.2	33.4	34.1	26.3	37.1	28.2
Stroke volume (ml)	23.9	12.2*	21.5	15.4	16.4	12.4
Shortening %	12.5	9.2	13.2	10.1†	13.2	9.3

* $P < 0.05$ paired *t* test control vs. dilution.
† $P < 0.05$ one-way analysis of variance albumin dilution vs. other dilutions.

TABLE II

Oxygen dynamics before and after hemodilution.

	Albumin		Unmodified hemoglobin		Modified hemoglobin	
	Control	Dilution	Control	Dilution	Control	Dilution
Arterial oxygen content (ml/dl)	11 ± 2	5 ± 1*	11 ± 1	8 ± 1	11 ± 1	9 ± 1*
Myocardial oxygen consumption (ml/min/100 g)	8 ± 5	3 ± 1	7 ± 1	6 ± 4	6 ± 1	7 ± 3
Arterial-coronary sinus oxygen difference (ml/dl)	5 ± 2	2 ± 1	3 ± 1	2 ± 1	3 ± 1	2 ± 1
Coronary sinus PO_2 (torr)	44 ± 11	45 ± 9	52 ± 14	43 ± 12	46 ± 8	41 ± 4
Lactate extraction	1 ± 8	2 ± 4	2 ± 4	0 ± 8	1 ± 5	1 ± 2

* $P < .05$ paired t-test comparing dilution to control; † $P < .05$ albumin dilution vs. other dilutions

tents before and after hemodilution. As can be seen from the hematocrit values, we achieved an approximate 50% exchange transfusion in each group. P50 values dropped significantly in both hemoglobin solution groups as a result of hemodilution with hemoglobin having a lowered P50. The post-dilution P50 values for the albumin group were unchanged from control and significantly higher than the post-dilution values with both hemoglobin solutions since the albumin group did not have any dilution of the native hemoglobin with hemoglobin having differing P50 values. Although the P50 of the diluting modified hemoglobin solution was greater than the unmodified solution (24 torr vs. 15 torr), this differences was not statistically discernable following the 50% *in vivo* exchange.

Myocardial function as characterized by stroke volume and percent shortening revealed a statistically significant decrease in every group and no differences between groups could be demonstrated.

Coronary blood flow was noted to increase in all three groups following exchange but the wide variability precluded statistical verification of this increase. Even if the wide variation were ignored and mean value comparisons were made between groups, there was no noticeable effect on utilizing hemoglobin solution as the diluting solution when compared to utilization of albumin solution.

Oxygen dynamics

The presence of a higher arterial oxygen content following hemodilution with two hemoglobin solution groups in comparison with the albumin group was the only statistically demonstrated benefit of hemodilution with hemoglobin solutions. Mean myocardial oxygen consumption values suggested that oxygen consumption was better maintained with hemoglobin solutions, but the wide variation again made statistical verification impossible. Coronary sinus pO_2 and lactate extraction were essentially unchanged following hemodilution with any of the three solutions.

EXERCISING DOG STUDIES

METHODS

Twenty mongrel dogs (30-45 Kg) of either sex previously trained to run on a treadmill were used. Following anesthesia with sodium pentobarbitol (25 mg/Kg) arterial and venous catheters were placed in the carotid and jugular vein for sampling purposes and the

spleens were removed. The animals were allowed to recover for two weeks before experimentation began. At this time, control measurements were collected during rest, exercise, and recovery periods. These measurements included EKG evaluated heart rate and arterial and venous blood samples for blood gas determination, hematocrit levels, arterial and venous oxygen content levels and venous lactate levels. All exercise data and blood samples were collected after the dogs had completed four minutes of exercise including two minutes at 2 mph and two minutes at 4 mph. All recovery data was collected ten minutes post exercise.

Following these control measurements, four groups of five animals each were exchange transfused with either their own blood (Sham exchange), 7% albumin, modified stroma-free hemoglobin solution, or unmodified stroma-free hemoglobin solution to an average hematocrit level of 15% (except for the Sham exchanged animals whose hematocrit level remained the same). Exchange volume was approximately 1.2 liters per dog and required approximately one hour to complete. Next, dogs had two liters of their own blood withdrawn and retransfused in a manner identical to the animals being exchanged with an exogenous solution.

Following transfusion, animals were again placed on the treadmill and the measurement sequence previously obtained was repeated. Additional measurements consisting of hematocrit, arterial oxygen content, total run time, and heart rate during rest, exercise and recovery were collected 24 hours, 48 hours, and 7 days post-transfusion.

Unmodified stroma-free hemoglobin solution was prepared in our laboratory in a manner previously reported[1,2,3,4]. The average P_{50} of this solution was 11 torr. Modified stroma-free hemoglobin solution was also prepared in our laboratory according to the method of Greenburg[5]. The P_{50} of this solution was approximately twice that of unmodified stroma-free hemoglobin solution or 22 torr. Seven percent albumin solution was prepared using bovine serum albumin, Fraction Five (Calbiochem, La Jolla, California). All P_{50} measurements were accomplished using a Radiometer Dissociation Curve Analyser (model DCA1). Blood gas results were obtained using model 813 Blood Gas Analyzer (Instrumentation Laboratories, Inc., Lexington, MA). Arterial and venous oxygen content results were obtained using an IL 282 CO-Oximeter. Venous lactate levels were determined according to the technique of Beutler[6]. All exercise runs were performed on a standard clinical treadmill starting at 0 grade and progressing in speed and grade. A complete exercise run consisted of completing two minute runs at 2, 4 and 6 miles per hour at 0 grade followed by four minute runs at 8 miles per hour at a 0, 3, and 5% grade. Total run time was measured in minutes with a completed exercise run taking 18 minutes.

Statistical analysis consisted of using Analysis of Variance, 1 way, followed by a Newman-Keuls Multiple Range Test.

Results

All animals receiving either a Sham exchange or having an exchange with either of the hemoglobin solutions ran at levels approaching control, as opposed to albumin-exchanged animals who were significantly limited ($p > 0.05$) in their exercise capabilities. Only two of five albumin animals were capable of exercising following exchange, whereas all animals in the other groups were capable of exercise.

After 24 hours, unmodified stroma-free hemoglobin solution animals experienced a significant drop ($p < 0.05$) in total run time compared to animals receiving modified stroma-free hemoglobin solution or animals receiving a Sham exchange. All albumin animals were capable of exercising at the 24 hour mark and increased their average running time from 2.3 to 9.7 minutes. Despite this increase, albumin and unmodified stroma free hemoglobin solution animals had a total exercise time which was still statistically lower than the modified stroma-free hemoglobin solution group. After seven days, all animals were able to recover sufficiently to approach control levels.

All animals were exchange to similar hematocrit levels, and by seven days post exchange, all but the albumin group had returned to a level close to control levels. Heart rate results during rest, exercise and recovery were obtained over the seven day observation period. Albumin animals, immediately following exchange while still at rest, had significantly higher heart rates compared to all other animals (194 ± 44 vs. 106 ± 25, 130 ± 20 and 116 ± 10 beats per minute, $p < .05$).

Arterial oxygen content results obtained immediately following exchange, showed that all three non-Sham groups experienced significant drops in oxygen content when compared to the Sham exchan-

ged animals ($p < 0.01$). In addition, the oxygen content of the albumin exchanged animals was significantly lower than either of the two hemoglobin groups (6.4 ± 1.2 vs. 10.4 ± 1.4 and 10.9 ± 0.9 ml O_2 dL, $p < 0.05$). Through the 48-hour period, there was a slight decrease in arterial oxygen content for both hemoglobin groups while the oxygen content of the albumin animals increased slightly so that the three groups were indistinguishable. By day seven, however, both hemoglobin groups had increased their oxygen content levels back to control values while the albumin group although continuing to increase slowly had values that were once again significantly lower than all three other groups ($p < .05$). No significant changes were detected in arterial-venous oxygen content values during the resting conditions. However, during exercise and recovery the two albumin-exchanged animals capable of exercise differed from the other three groups with lower mean arterial-venous oxygen content differences.

Albumin exchanged animals at rest had significantly higher lactate levels compared to all other groups (4.5 ± 3.0 vs. 1.4 ± 0.4, 1.7 ± 1.4 and 1.3 ± 2.0 mM L, $p < 0.05$). Lactate levels during exercise and recovery for the two albumin dogs capable of exercising following exchange, were higher than levels found for either hemoglobin solution, but the small sample size of two limits our ability to compare these results on a statistical basis. Lactate levels for both hemoglobin solutions did not differ significantly from the Sham values for either rest, recovery, or recovery following exchange.

Exercising swine studies

Methods

Fourteen swine (46-53 Kg) of either sex were chronically instrumented in a manner similar to that previously reported[29]. The surgical preparation was performed under anesthesia consisting of induction with ketamine (1 mg/Kg IM) and surital (20 mg Kg, IV), and maintenance with a combination of 0.5% halothane with oxygen and intravenous succinylcholine (400 mg/L at 7 ml min). A left lateral thoracotomy was performed through the fourth intercostal space and the pericardium was opened. Left ventricular internal diameter ultrasonic dimension crystals were implanted as indicators of global heart mechanics. These internal diameter crystals were positioned by pulling one crystal with its wire through the left ventricular lateral wall with a large needle in the manner described by Bishop[30]. The crystal remained in the left ventricular chamber against the septum while the lead wires continued out through the septum and right ventricle. The second crystal was placed on the endocardium of the lateral wall through the track created by the passage of the first crystal. Silastic catheters (.085" ID) were placed in the descending thoracic aorta (to monitor pressure, collect blood samples for blood gas and oxygen content analysis, and to serve as a port for the withdrawal of blood samples during microsphere injections), the pulmonary artery (to obtain a mixed venous sample for blood gas and oxygen content analysis), and the left atrium (to monitor pressure, and to inject microspheres). An 18 mm (internal diameter) electromagnetic flow probe (Biotronix Silver Springs, MD) was placed around the ascending aorta to measure output (CO_2) and ejection velocity.

All catheters and lead wires were brought out of the thoracic cavity via the fourth intercostal space and then run subdermally to the back, where they were externalized.

Myocardial function

Ventricular dimensions were measured using implanted ultrasonic crystals connected to a sonomicrometer (S.A.). Changes in internal diameter during ventricular ejection (ΔD) were defined as EDD-ESD × 100 divided by EDD where EDD is end-diastolic diameter and ESD is end-systolic diameter. EDD was defined as the time coincident with the peak of the R wave of the EKG and ESD as the time point of minimal chamber diameter.

Regional blood flow

Distribution of cardiac output was determined by injection of carbonized microspheres (15 microns) in a manner previously reported[31]. Regional blood flow was calculated by the method described by Domenech[32] allowing flows to be expressed in ml min g tissue (wet weight). Tissue blocks of approximately 5 grams were taken from the brain, epicardium, endo-

cardium, kidney, spleen, intestine, stomach, and skeletal muscle regions. These samples were minced dried, and counted in a Packard-Auto Gamma Spectrophotometer model 5912 equipped with a multichannel analyzer. Analysis of the energy spectra was performed according to the matrix inversion method of Schosser[15].

Blood gas analysis and oxygen content determinations were derived from blood samples taken from the arterial and venous catheters just prior to microsphere injection.

Total oxygen consumption was defined as arterial-venous oxygen content difference × cardiac output (ml/kg min). Total oxygen transport (ml O_2/kg min) was calculated from arterial oxygen content × cardiac output. Lactate determinations were done using the technique of Beutler[16]. Microspheres detected in the lungs provided data for the percent of cardiac output shunted around the capillary beds[17].

Animal protocol

Familiarization of the animals with treadmill exercise was carried out in the manner previously reported by this laboratory[18]. Two weeks following surgical instrumentation, each animal was run twice for different days on a treadmill to a state of exhaustion for determination of maximal heart rate. Exhaustion was considered to be reached when the animal could no longer maintain the workload imposed upon it.

When the peak rate was reached and the animal began to falter, final measurements were made. This was described as a maximal exercise state. Exercise capacity was measured in total time to exhaustion (minutes) and in total work performed (kg meters).

Experimental design

Four conditions were studied in each animal. Control data for the resting state was collected with the animal standing quietly on the treadmill. These measurement procedures consisted of recording hemodynamic measurements, taking arterial and venous blood samples, and injecting a dose of tracer microspheres into the left atrial catheter. These procedures were repeated during maximal exercise conditions. Following a 30 minute recovery, at which time the normal resting conditions were re-established, each animal was exchanged to an average hematocrit level of 15% with either hemoglobin solution or albumin solution. Exchange time was 1.5 hours. Post-exchange control and maximal exercise measurements were made. The following measurements were obtained at each recording: cardiac output, arterial and left atrial pressure, heart rate, stroke volume, change in flow per unit time (dF/dt), and sonomicrometry-measured ventricular dimensions.

Data analysis and solutions used

All statistics were performed using one way analysis of variance with a Newman-Keuls multiple range test. Group analysis between the four resting conditions and the four exercise conditions was calculated.

Two animals died following albumin exchange with ventricular fibrillation during exercise and could not be included in this study. Therefore, six animals were used in both the stroma-free hemoglobin solution and in the albumin solution groups.

Two experimental solutions, bovine serum albumin and unmodified stroma-free hemoglobin solution, were prepared for these experiments. The albumin solution was prepared using serum bovine albumin as previously described. This bovine albumin was suspended in hemodialysis fluid to provide a final concentration of approximately 7 mg/DL. The stroma-free hemoglobin solution was prepared using a modification of the technique described by Greenburg[19] and also was prepared in order to provide a final concentration of approximately 7 mg/DL of hemoglobin.

RESULTS

All results are shown in tables III-VI. As can be seen in table III, control animals in the two groups had hematocrits of approximately 30%, which increased slightly with exercise. Following hemodilution with either albumin or stroma-free hemoglobin solution, resting hematocrits of 14% increased to 18% with exercise.

Albumin exchanged animals

Resting condition: Arterial oxygen content and A-V O_2 difference was significantly lower than control following exchange-transfusion with albumin (tab. III). Oxygen consumption was not compromised due to increased cardiac output, heart rate, and dF/dt (tab. IV). Lactate production did not increase significantly (table III). An increase in coronary blood flow was detected, but there were no changes in cerebral or visceral organ blood

TABLE III

Hemodynamic measurements comparing albumin with stroma free hemoglobin solution

	Albumin				Stroma free hemoglobin solution			
	Control		Exchange		Control		Exchange	
	Rest	Exercise	Rest	Exercise	Rest	Exercise	Rest	Exercise
Heart rate (beat/min)	113 ± 10	257 ± 18	151 ± 21*	231 ± 20*	123 ± 22	262 ± 20	108 ± 13	258 ± 14
Cardiac output (ml/min/kg)	116 ± 31	263 ± 85	179 ± 45*	264 ± 57	98 ± 19	211 ± 36	99 ± 13	251 ± 37
Stroke volume (ml)	41 ± 7	42 ± 13	45 ± 12	49 ± 13	34 ± 10	33 ± 6	39 ± 10	40 ± 7
Left atrial pressure (mmHg)	6.4 ± 3.6	12.6 ± 3.7	8.4 ± 4.7	16.3 ± 6.0	5.5 ± 4.9	11.0 ± 7.0	9.0 ± 7.6	13.0 ± 8.9
Aortic pressure (mmHg)	114 ± 9	138 ± 12	97 ± 19*	107* ± 12*	105 ± 15	127 ± 13	123 ± 15	150 ± 22*
dP/dt	7.8 ± 1.7	17.6 ± 5.2	11.8 ± 3.3*	13.4 ± 1.8	7.2 ± 1.2	16.0 ± 2.9	7.4 ± 1.6	15.7 ± 3.6

* $P < 0.05$ exchange vs. control conditions
* $P < 0.05$ exchange vs. rest conditions

flows (tab. V). Myocardial function, as measured by percent change in diameter, was not compromised (tab. VI).

Exercising condition: Arterial oxygen content, arterial-venous oxygen content difference, oxygen consumption, total oxygen transport, and aortic pressure were decreased significantly when compared to control exercise conditions (tabs. III, IV). Despite a decrease in heart rate, cardiac output remained unchanged because stroke volume increased. Both lactate production and left atrial pressure showed increases compared to control exercise, while aortic pressure was significantly lower (tabs. III, IV). The albumin exchange resulted in significant increases in both coronary and cerebral blood flow, as well as decreases in mean visceral organ flow (tab. V). Albucin exchanged animals could only run at about 50% exercise capacity of their control exercise capacity, but this decreased performance was not significantly different from the 60% achieved by the hemoglobin animals (tab. VI).

Stroma-free hemoglobin solution

Resting condition: As noted following albumin exchange, stroma-free hemoglobin solution exchange-transfused animals had a significant drop in arterial oxygen content. This decrease was not of the magnitude found following albumin exchange. Despite this drop, no changes occurred in oxygen consumption, cardiac output, heart rate, lactate production (tabs. I, II), or organ blood flow (tab. III). As with the albumin-exchanged animals, myocardial function was not changed (tab. IV).

Exercising condition: During exercise, both arterial oxygen content and arterial-venous oxygen content differences were significantly lower than control exercise values (tab. III). Cardiac output remained similar to control levels (tab. V), but unlike the albumin animals, the stroma-free hemoglobin solution animals showed no changes in oxygen consumption, oxygen transport, lactate production, heart

TABLE IV

Oxygen dynamic measurements comparing albumin solution versus stroma free hemoglobin solution

	Albumin				Stroma free hemoglobin solution			
	Control		Exchange		Control		Exchange	
	Rest	Exercise	Rest	Exercise	Rest	Exercise	Rest	Exercise
Hematocrit (%)	31 ± 4	36 ± 3	14 ± 3**	18 ± 4**	30 ± 4	33 ± 3	14 ± 1**	18 ± 1**
Arterial oxygen content (ml/dl)	12.7 ± 2.5	13.8 ± 3.2	6.2 ± 1.8	6.6 ± 1.1*	14.0 ± 2.7	15.6 ± 2.4	9.8 ± 1.1**	11.0 ± 1.7**
Arterial-venous oxygen content difference (ml/dl)	6.1 ± 2.6	19.7 ± 1.3	3.5 ± 1.9*	5.5 ± 1.5*	7.7 ± 2.5	12.2 ± 1.7	5.9 ± 0.6	9.3 ± 1.5*
Oxygen consumption (ml/kg/min)	6.8 ± 1.9	27.4 ± 8.3	6.4 ± 2.4	16.5 ± 5.4**	7.4 ± 2.3	25.7 ± 5.2	5.9 ± 1.2	23.9 ± 6.3
Oxygen transport (ml/kg/min)	14.5 ± 3.9	35.1 ± 11.2	11.3 ± 4.6	17.3 ± 4.3*	13.8 ± 3.9	33.2 ± 8.9	9.7 ± 1.9	28.1 ± 7.4
Lactate (mM/L)	0 ± 0	1.7 ± 2.2	2.8 ± 2.2	23.5 ± 4.3**	0.7 ± 0.9	16.0 ± 3.0	2.0 ± 3.0	20.4 ± 4.5
Venous PO_2 (mmHg)	37 ± 2	20 ± 6	33 ± 6	33 ± 9	32 ± 6	21 ± 4	26 ± 6	28 ± 15

* $P < 0.05$ albumin exchange versus others.
** $P < 0.05$ exchange versus control

rate, or dF/dt (tab. IV). In addition, these animals showed no detectable changes in organ blood flow (tab. V).

Microsphere shunting

The results of the shunting measurements are shown in table V. With stroma-free hemoglobin solution and albumin solution, the shunt was increased significantly at rest and decreased significantly with exercise. Albumin and stroma-free hemoglobin solution animals appeared to affect microsphere shunting in a similar fashion.

DISCUSSION

Each of our three studies was designed to examine different physiological considerations in attempting to establish both the efficacy and freedom from toxicity of the two basic hemoglobin solutions (modified and unmodified). Since the studies were designed with separate objectives, each will be discussed separately.

SWINE RIGHT HEART BYPASS STUDIES

In performing hemodilution on cardiopulmonary bypass at a 50 % exchange level, we noted generally satisfactory maintenance of myocardial function, although all groups were noted to have statistically verifiable decreases in stroke volume. Previous experience with this animal model indicated generally well preserved myocardial function if the hematocrit was kept at 15 % or greater[26]. That same report documented a loss of myocardial

TABLE V

Blood flow measurements (ml min 100 g tissue) comparing albumin solution vs stroma free hemoglobin solution

	Albumin				Stroma free hemoglobin solution			
	Control		Exchange		Control		Exchange	
	Rest	Exercise	Rest	Exercise	Rest	Exercise	Rest	Exercise
Coronary	100 ± 29	318 ± 78	318 ± 167*	652 ± 182**	106 ± 30	424 ± 139	178 ± 59	568 ± 109
Brain	49 ± 10	49 ± 12	55 ± 13	97 ± 25*	42 ± 11	48 ± 13	51 ± 11	69 ± 23
Skeletal muscle	9 ± 9	42 ± 35	9 ± 14	76 ± 84	4 ± 3	46 ± 24	4 ± 3	80 ± 33
Kidney	244 ± 90	58 ± 58	273 ± 107	5 ± 5	161 ± 97	55 ± 57	139 ± 102	70 ± 103
Intestine	41 ± 27	33 ± 9	44 ± 27	2 ± 2	34 ± 34	12 ± 15	37 ± 36	14 ± 18
Stomach	16 ± 9	2 ± 11	18 ± 13	0.3 ± 0.3	17 ± 14	12 ± 11	20 ± 11	6 ± 10
Liver	31 ± 3	19 ± 17	31 ± 24	9 ± 15	26 ± 18	17 ± 11	18 ± 7	16 ± 22
Spleen	332 ± 137	64 ± 73	255 ± 273	3 ± 6	222 ± 90	48 ± 73	139 ± 82	
Endo Ep.	1.2 ± 0.2	2.1 ± 1.1	1.0 ± 0.3	0.8 ± 0.2	1.0 ± 0.3	0.9 ± 0.2	1.2 ± 0.2	1.1 ± 0.2
Lung (% shunt)	4.7 ± 1.9	8.5 ± 3.2	9.3 ± 2.7**	1.95 ± 0.5***	4.2 ± 1.0	1.3 ± 0.4***	7.2 ± 1.3	1.1 ± 0.5**

Lung measurements are represented by % peripheral blood shunting.
* $P < 0.025$, exchange versus others
** $P < 0.025$, exchange versus control
*** $P < 0.05$, exercise versus rest

TABLE VI

Exercise performance and myocardial function measurements comparing albumin vs stroma free hemoglobin solution

	Albumin				Stroma free hemoglobin solution			
	Control		Exchange		Control		Exchange	
	Rest	Exercise	Rest	Exercise	Rest	Exercise	Rest	Exercise
Total work (kg meter)		1942 ± 535		981 ± 297*		2052 ± 278		1245 ± 294*
Time to exhaustion (min)		11.4 ± 2.2		5.7 ± 1.2*		12.1 ± 2.2		7.3 ± 1.8*
% diameter shortening	26 ± 11	27 ± 12	27 ± 10	25 ± 8.5	29 ± 6	32 ± 9	25 ± 1	35 ± 7
End-diastolic diameter (mm)	22 ± 3	22 ± 4	22 ± 3	22 ± 3	21 ± 2	20 ± 3	22 ± 0.4	19.5 ± 2

* $P < 0.005$, exchange vs. control

function if the hematocrit was at 10 % or less. The increased oxygen capacity provided by the hemoglobin was insufficient to prevent the decrease in stroke volume that occurred when the hematocrit reached approximately 12 %. Myocardial contractile function may not be a sensitive indicator of the benefits of increasing oxygen capacity as other studies examining myocardial function in nonanesthetized animals also failed to show any benefit when stroma-free hemoglobin solution was used [ ]. Given the 50 % exchange used in this study, it was not possible to demonstrate any phsyiologic benefits from utilization of stroma-free hemoglobin solution having improved oxygen offloading characteristics. At higher levels of exchange, we have demonstrated previously a physiologic effect when blood of differing P_{50} characteristics was used [ ]. However, we must conclude that under the conditions of cardiopulmonary bypass, when the degree of exchange is at 50 %, there were no demonstrable physiologic benefits from the usage of stroma-free hemoglobin solution as opposed to albumin solution. This data does not, however, contradict our earlier study evaluating stroma-free hemoglobin solution as a cardiopulmonary bypass prime at extremely low hematocrit levels [ ]. In that study utilizing a hematocrit of 5 %, we were unable to maintain any cardiac function unless the albumin prime was replaced with a stroma-free hemoglobin solution prime.

Stroma-free hemoglobin solution is not the only oxygen-carrying solution being evaluated as a blood substitute and consequently as a cardiopulmonary bypass priming solution. Fluorocarbon emulsions have been evaluated in this role, primarily by Engelman [ ]. He noted that these emulsions were generally effective, but also reported some disturbing increases in pulmonary vascular resistance, thus raising concern regarding the safety and tolerance of these solutions.

Concern regarding non-nephrotic toxicity of the hemoglobin solutions continues to be raised, however we failed to document any measurable toxicity in this perfused swine model. Stroma-free hemoglobin solution is not a manufacturable « chemical » solution such as fluorocarbon emulsions, and is subject to preparation variations that may result in toxic sequelae when the solutions are administered to animals models. Obviously, a stroma-free hemoglobin solution intended for human use must have sufficient quality control and purity to avoid any toxic reaction.

Exercising dog studies

Ample scientific literature has established the ability of stroma-free hemoglobin solution to maintain gross physiologic oxygen requirements [ ]. However, little has been done to investigate the effect of the relatively limited intravascular retention time of these solutions and their subsequent ability to act as an adequate resuscitative fluid beyond the initial exchange, and even less is known about the effects of introducing conditions such as exercise.

Immediate post-transfusion period

All of our animals appeared well following exchange with any of the solutions, suggesting that none of the solutions were significantly toxic. However, the albumin animals were clearly more limited in their ability to exercise, an activity requiring additional oxygen. Albumin animals had significantly shorter total run times, higher resting heart rates, and higher resting venous lactate levels compared with either the hemoglobin group or the Sham group. The association of oxygen deficiency as the reason for these abnormalities is further supported by the albumin animals lower arterial oxygen content values when compared to other groups.

It clearly appears that both hemoglobin solutions were able to transport enough

oxygen to maintain exercise despite their differences in oxygen affinity. The more normalized P_{50} of the modified stroma-free hemoglobin solution did not provide any significant initial advantage over unmodified stroma-free hemoglobin solution as all results comparing these two solutions in the immediate post-exchange period were virtually identical. Furthermore, both solutions appeared capable of maintaining almost normal physiologic function as almost all of the values in the hemoglobin animals approached those achieved by the Sham dogs.

The lower arterial-venous oxygen content difference values noted in the albumin animals during exercise and recovery periods could be due to either an increased cardiac output or decreased oxygen consumption. Furthermore, the dogs had an increase in lactate levels during exercise, indicating that the decreased oxygen consumption was not adequately meeting oxygen requirements. These results would seem to support the thesis that it is the change in oxygen carrying capacity between the albumin and the stroma-free hemoglobin solution animals that is responsible for the observed physiologic differences.

Albumin animals 24 hours post-exchange had an exercise response that was still limited, but would show signs of recovery in terms of increased total run time, decreased heart rate, and slightly increased arterial oxygen content and hematocrit levels. It appears that normal metabolic and physiologic mechanisms were able to compensate for the limiting oxygen availability resulting from the albumin transfusion.

By day seven all groups were back to control levels in all categories except that the albumin exchanged animals still experienced a significantly lower arterial oxygen content level. This decreased oxygen content appeared to correlate with the more persistently depressed hematocrit level still present in the albumin animals by the seventh day. Perhaps the initial presence of stroma-free hemoglobin solutions may, in some way, accelerate the return of a more normal hematocrit as well as normal physiological functions, even though the stroma-free hemoglobin solution is no longer in the vascular bed.

This study appears to provide support for several conclusions. Moderate hemodilution with a more normalized P_{50} does not provide any initial advantage in terms of exercise capacity. Both hemoglobin solutions provide a significant advantage over 7 % albumin solution as a resuscitative fluid. However, these advantages appear to be short-lived and are of little benefit 48 hours post-exchange.

By the seventh post-transfusion day, all animal groups approach control levels and are virtually indistinguishable, although there is some evidence that the stroma-free hemoglobin solution groups may have obtained this control level at a slightly faster rate. The initial beneficial effects appear to be due to the increased oxygen carrying capacity of the hemoglobin solutions while the somewhat more subtle long-range benefits must be speculated as arising from an effect that outlast the actual presence of the solutions.

A final conclusion relates to our failure to document any significant non-stroma related toxicity reported by others. However, the recent documentation of toxicity by reliable investigators continues to be disturbing. When this information is combined with similar toxic reactions in humans [3] continued caution must be exercised in proposing these solutions for clinical applications.

Exercising swine studies

The results from this study indicate that exercise performance was maintained more effectively by hemodilution with unmodified stroma-free hemoglobin solution than with 7 % albumin. This is indicated by greater oxygen delivery, aortic pressure, exercise dF/dt, duration of exercise, and decreased lactate production in the stroma-free hemoglobin solution ex-

changed animals compared to the albumin-exchanged animals.

At rest following albumin exchange, oxygen consumption and oxygen transport remained close to control levels due to an increase in cardiac output. Because stroke volume remained constant, this increase in cardiac output was due to an increase in heart rate. This coincides with the results of other investigators [4, 5] who reported that cardiac output varied inversely with hematocrit primarily due to decreased viscosity and peripheral vascular resistance. Increased cardiac output was not detected in animals exchanged with stroma-free hemoglobin solution which has a viscosity similar to the viscosity of albumin solution [1]. Therefore, it may be that the increase in cardiac output is a combination of an autoregulatory mechanism in response to mild hypoxia as well as the decreased viscosity.

During exercise, albumin-exchanged animals had lower oxygen consumption when compared to pre-exchange exercise values. This indicates insufficient oxygen to meet the higher oxygen demands of exercise. This was associated with a significant decrease in total oxygen transport due to the decreased transport of oxygen with albumin solution and may also be due to the increased peripheral blood shunting found in these animals. Evidence for this shunting is based on the decreased aortic pressure and lower peripheral resistance noted in the albumin-exchanged animals. Previous work from this laboratory [1, 2] has shown that the decrease in absolute visceral blood flow is a sensitive indicator of exercise stress in the pig. This sensitivity is apparent when comparing the greater decrease in visceral blood flow in the albumin exchanged exercised group compared to the other exercised groups.

If the ultimate limit of aerobic exercise is the limitation of oxygen transport or oxygen diffusion as has been suggested by Blomquist and Saltin [3], then the redistribution of blood flow away from the viscera during exercise may be enhanced further during hemodilution and exercise.

The degree of shunting shown in these studies is low enough to be physiologically insignificant. During exercise, shunting decreases as might be expected since visceral blood flow also decreases with exercise and much of the shunting may be coming from visceral organs. Shunting seen at rest in our animals is similar to that seen in swine from other laboratories [4].

During exercise stress in the albumin-exchanged animals, heart rate did not increase cardiac output enough to compensate for the increased oxygen requirements. This may be explained by a failure to meet the necessary oxygen requirements needed to elicit a maximal heart rate. Further support for this interpretation is provided by the fact that albumin-exchanged animals ran for an average of 1.6 minutes less than stroma-free hemoglobin solution-exchange animals and approximately six minutes less than under control conditions

Previous reports [1] have shown an increase in coronary blood flow with hemodilution. We also noted that coronary blood flow was greater following hemodilution with albumin during rest and exercise. Since there was no increase in coronary blood flow following stroma-free hemoglobin solution exchange, this suggests that these animals were not experiencing as high a level of hypoxia.

The constancy of percent change of diameter and end-diastolic diameter suggests that despite decreased oxygen availability following exchange with albumin *versus* stroma-free hemoglobin solution, myocardial performance (determined by these indices) was not impaired. This apparent constancy of myocardial function may be deceiving, however, because we have little information regarding how long myocardial function could have been maintained under these conditions. In addition, albumin-exchanged exercised pigs experienced a significant decrease in aortic pressure and a marked increase in left atrial pressure during exercise. Such changes are often seen during initial stages of myo-

cardial failure [3]. Coronary blood flow for these animals may be below the reserve capacities of the heart [4].

Lactate production following albumin exchange and exercise increased significantly even though total exercise time was slightly less than with stroma-free hemoglobin solution. Cain reported that increased lactate can be the result of « hypoxemia produced by autoexchange anemia » [1]. Although we did not find increased lactate at rest following exchange, we did document increased lactate during exercise. Again, we are provided with evidence that albumin animals were experiencing a greater oxygen deficiency which ultimately led to a lower exercise capacity.

An issue not directly addressed in this study is that of the potential toxicity of the solutions. The major problem of tissue damage from red cell stroma has generally been solved, however some recently published articles have documented significant hemodynamic and hematological toxicity not associated with the stromal components [5, 6]. Some of the reported abnormalities such as minor coagulation defects would not be detected in our study, however the major hemodynamic problems, such as significant arrhythmias, hypoxia, and death, would have been detected. Since these problems were reported in rabbits rather than in pigs and dogs, it would seem reasonable to conclude that the animal model used to evaluate that solution is of crucial importance.

Conclusion

While the art and science of manufacturing stroma-free hemoglobin solution has not reached a level sufficient to provide a product that can function as a complete red cell substitute, we feel these studies demonstrate an improvement in various physiologic parameters even when an incomplete solution is used. Furthermore, we were impressed that these beneficial effects were apparent at moderate levels of hemodilution, although the imposition of an exercise stress was essential to elucidate some of these differences.

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References

1. Bonhard K.: *Acute oxygen supply by infusion and hemoglobin solutions.* Fed Proc., *34*, 1466-467, 1975.
2. Moss G. S., DeWoskin R., Rosen A. L., Levine H., Palani C. K.: *Transport of oxygen and carbon dioxide by hemoglobin saline solution in the red cell free primate.* Surg. Gynecol. Obstet., *142*, 357-362, 1978.
3. Devenuto F., Friedman T. I. Neville J. R., Peck C. C.: *Appraisal of hemoglobin solutions as blood substitutes.* Surg. Gynecol. Obstet., *149*, 417-436, 1979.
4. Sasuzuhn T.: *Studies of hepatoglobin. I. Immunochemical properties of heptaglobin and antihemoglobin antibody.* Proc. Jap. Acad., *46*, 820, 1970.
5. Cochin A., Das Gupta T. K., Dwoskin R., Moss G. S.: *Immunogenic properties of stroma vs. stroma-free hemoglobin solution.* Surg. Forum., *23*, 19-21, 1972.
6. Rabiner S. F.: *Evaluation of a stroma-free hemoglobin solution for use as a plasma expander.* J. Exp. Med., *12*, 1127-1142, 1967.
7. Rabiner S. F.: *Hemoglobin solution as a plasma expander.* Fed. Proc., *34* (6), 1454-1457, 1975.
8. Usami S., Chien S., Gregerson M. I.: *Hemoglobin solution as a plasma expander. Effects on blood viscosity.* Proc. Soc. Exp. Biol. Med., *136*, 1232-1235, 1971.
9. Moores W. Y., Devenuto F., Heydorn W. H., Weiskopf R. B., Baysinger B. S., Greenburg A. G., Utley J. R.: *Extending the limit of hemodilution on cardiopulmonary bypass using stroma-free hemoglobin solution.* J. Thorac. Cardiovasc. Surg., *81*, 155-162, 1981.

10. GREENBURG A. G., ELIA C., LEVINE B., BELSHA J., PESKIN G. W.: *Hemoglobin and the oxyhemoglobin dissociation curve.* J. Trauma, 5, 943-950, 1975.

11. GREENBURG A. G., PESKIN G. W., HOYT D. B., MOORES W. Y.: *Is it necessary to improve the intravascular retention of hemoglobin solutions?* Crit. Care Med., *10*, 266-269, 1982.

12. GREENBURG A. G., SCHOOLEY M., PESKIN G. W.: *Improved retention of stroma-free hemoglobin solution by chemical modification.* J. Trauma, *17*, 501-504, 1977.

13. PESKIN G. W., O'BRIEN K., RABINER S. F.: *Stroma free hemoglobin solution. The «ideal» blood substitute?* Surgery, *66*, 185-193, 1969.

14. RABINER S. F., O'BRIEN K., PESKIN S. B., FRIEDMAN L. H.: *Further studies with stroma-free hemoglobin solution.* Ann. Surg., *171*, 615-622, 1970.

15. WHITE C. T., MURRAY A. J., SMITH D. J., GREENE J. R., BOLIN R. B.: *Synergistic toxicity of endotoxin and hemoglobin.* J. Lab. Clin. Med. (in press, 1986).

16. WHITE C. T., MURRAY A. J., GREENE J. R., SMITH D. J., MEDINA F., MAKOVEC G. T., MARTIN E. J., BOLIN R. B.: *Toxicity of human hemoglobin solution infused into rabbits.* J. Lab. Clin. Med. (in press, 1986).

17. KOTHE N., EICHENTOPF B., BONHARD K.: *Characterization of a modified stroma-free hemoglobin solution as an oxygen-carrying plasma substitute.* Surg. Gynecol. Obstet., *161*, 563-569, 1985.

18. SANDERS M., WHITE F. C., BLOOR C. M.: *Cardiovascular responses of dogs and pigs exposed to similar physiologic stress.* Comp. Biochem. Physiol., *58*, 365-370, 1979.

19. BISHOP V. B., HORWITZ L. D., STONE H. L., STEGAL H. F., ENGELKER E. J.: *Left ventricular internal diameter and cardiac function in unconscious dogs.* J. Appl. Physiol., *5*, 619-623, 1969.

20. SAVAGE R. M., GUTH B. S., WHITE F. C., HAAN A. D., BLOOR C. M.: *Correlation of regional myocardial blood flow and function with myocardial infarct size during acute myocardial ischemia in the conscious pig.* Circulation, *64*, 699-707, 1981.

21. DOMENECH G. J. HOFFMAN J. I. E., NOBLE M. I. M., SAUNDERS K. B., HENSON J. R., SUBIJANTO S.: *Total and regional coronary blood flow measured by radioactive microspheres in conscious and anesthetized dogs.* Circ. Res., *25*, 581-596, 1969.

22. SCHOSSER R., ARFORSKE E., MESSMER K.: *Mic-II-a program for the determination of cardiac output, arterio-venous shunt, and regional blood flow using the radiomicrosphere method.* Comp. Programs Biomed., *9*, 19-38, 1979.

23. BEUTLER E.: *Red Cell Metabolism. A Manual of Biochemical Methods.* Grune & Stratton, New York, 1971, p. 108.

24. HEYMANN M. A., PAYNE B. D HOFFMAN J. I. E., RUDOLPH A. M.: *Blood flow measurements with radionuclide-labeled particles.* Prog. Cardiovasc. Dis., *30*, 55-79, 1977.

25. SANDERS M., WHITE F. C., PETERSON T. M., BLOOR C. M.: *Characteristics of coronary blood flow and transmural distribution in miniature pigs.* Am. J. Physiol., *235* (Heart. Cir. Physiol., 4): H601-H609, 1978.

26. MOORES W., HEYDORN W., DEMBITSKY W., RAYSINGER M., WILLFORD D., MAHONEY E.: *Hemodilution and cardiopulmonary bypass. Support of the pig heart at a reduced threshold hematocrit of 15 %.* Circulation, 65 (Supp. II), 305, 1982.

27. MOORES W. Y., WHITE F. C., BLOOR C., GREENBURG A. G., MACK R., WILLFORD D. C.: *The physiologic effect of oxygen transport by hemoglobin solutions.* In: *Advances in Blood Substitute Research*, edited by Bolin, Geyer, Nemo. Alan R. Liss, New York, 1983, pp. 88-99.

28. MOORES W. Y., WILLFORD D. C., CRUM J. D. NEVILLE J. R., WEISKOPF R. B., DEMBITSKY W. P.: *Alteration of myocardial function resulting from changes in hemoglobin oxygen affinity.* Circulation, *58* (Supp. II), 225, 1978.

29. MOORES W. Y., DEVENUTO F., HEYDORN W. H et al.: *Effectiveness of stroma-free hemoglobin solution as seen in a right heart bypass swine model.* Crit. Care Med., *10*, 279-282, 1982.

30. ENGELMAN R. M., ROUSOU J. H., DOBBS W. A.: *Fluorsol-DA. An artificial blood for total cardiopulmonary bypass.* Ann. Thorac. Surg., *32*, 528-535, 1981.

31. ENGELMAN R. M., DOBBS W. A., ROUSOU J. H., ANTSIMOWICZ L.: *Use of fluorsol-DA during open heart surgery.* In: Advances in Blood Substitute Research, edited by Bolin, Geyer, Nemo. Alan R. Liss, New York, 1983, pp. 273-282.

32. DEVENUTO F., MOORES W. Y., ZEGNA A. I., ZUCK T. F.: *Total and partial blood exchange in the rat with hemoglobin prepared by crystallization.* Transfusion *17*, 655-662, 1977.

33. SAVITSKY J. P., DOCZI J., BLACK J. ARNOLD J. D.: *A clinical safety trial of stroma-free hemoglobin.* Clin. Pharmacol. Ther., *23*, 73, 1978.

34. CAIN S. M.: *Appearance of excess lactate in anesthetized dogs during anemic and hypoxic hypoxia.* Am. J. Physiol., *209*, 604-610, 1965.

35. JAN K. M., CHIEN S.: *Effect of hematocrit variations on coronary hemodynamics and*

oxygen utilization. Am. J. Physiol., 233, H106-H113, 1977.

36. DEVENUTO F.: *Viscosity of human blood hemodiluted with crystalline hemoglobin solution.* Transfusion, *21*, 752, 1981.
37. BLOMQVIST C. G., SALTIN B.: *Cardiovascular adaptations to physical training.* Ann. Rev. Physiol., *45*, 169-189, 1983.
38. HOI R. P., WYLER F., STALDER G.: *Validation studies for the use of microsphere methods in cats and young mini pigs.* Basic. Res. Cardiol. *75*, 747-756, 1980.
39. THEROUX P., ROSS J. Jr., FRANKLIN D., COVELL J. W., BLOOR C. M., SAGAYAMA S.: *Regional myocardial function and dimension early and late after myocardial infarction.* Circ. Res., *40*, 158-165, 1977.
40. WHITE F. C., SANDERS M., BLOOR C. M.: *Coronary reserve at maximal heart rate in the exercising swine.* J. Cardiac. Res., *1*, 31-40, 1981.
41. WHITE C. T., MURRAY A. J., SMITH D. J., GREENE J. R., BOLIN R. B.: *Synergistic toxicity of endotoxin and hemoglobin.* J. Lab. Clin. Med. (in press, 1986).
42. WHITE C. T., MURRAY A. J., GREENE J. R., SMITH D. J., MEDINA F., MAKOVEC G. T., MARTIN E. J., BOLIN R. B.: *Toxicity of human hemoglobin solution infused into rabbits.* J. Lab. Clin. Med. (in press, 1986).

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IMPROVED EXERCISE PERFORMANCE IN HEMODILUTED PIGS WITH STROMA-FREE HEMOGLOBIN SOLUTION

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Running Head: Exercise Performance with Hemoglobin Solution

ABSTRACT

We exchange-transfused 14 awake swine (sus scroffa) to a hematocrit of 14% with either 7% bovine serum albumin or 7% human stroma-free hemoglobin solution (SFHS) and exercised them on a treadmill. The albumin solution animals showed a significant decrease in arterial oxygen content that resulted in a decrease in arterio-venous oxygen content difference and an increase in cardiac output at rest. During exercise, these animals showed significant decreases in aortic pressure, arterial oxygen content and arterio-venous oxygen content difference compared to control exercise. This was not compensated for by increased cardiac output and resulted in decreased oxygen consumption, duration of exercise capacity, and total work performed. These animals also had increases in lactate production, cerebral and coronary blood flows, and left atrial pressure, and marked decreases in visceral organ blood flow and dF/dt compared to pre-exchange exercise. In addition, two animals died with ventricular fibrillation following albumin exhange and maximal exercise. Animals hemodiluted with SFHS showed decreased arterial oxygen content and a decrease in arterio-venous oxygen content difference during exercise, but otherwise had physiologic data similar to non-exchange control conditions. The higher oxygen content provided by the SFHS allowed the anemic pigs to approach their non-anemic condition.

Index Terms: Anemia, hemodynamics, blood flow distribution, oxidative metabolism

INTRODUCTION

Reduction in the circulating hematocrit either as a result of blood loss or erythrocyte destruction is a common clinical situation. Although the restoration of a normal hematocrit and normal oxygen capacity would seem to be a desirable goal in most cases, it is usually not possible to achieve this goal on an acute basis without subjecting patients to the risks of blood transfusion. In addition, a reduced hematocrit may actually provide a beneficial effect in certain clinical settings by decreasing blood viscosity. Currently, this is only possible by compromising the oxygen carrying capacity. Colloidal or crystalloid cell-free resuscitation fluids, capable of carrying only dissolved oxygen, result in a decreased arterial oxygen content. Stroma-free hemoglobin solution (SFHS) has been considered as a possible hemodiluting vehicle that maintains oxygen capacity, decreases viscosity, and avoids the risk of homologous red blood cell transfusions. Previously, these solutions have been examined primarily at extreme levels of anemia (6,22), in anesthetized animals (18), or in conscious animals at rest (20).

The concept that a cell-free artificial blood could provide increased O_2 carrying capacity above that of dissolved oxygen in a crystalloid solution dates back to 1934 (1) with the development of a true stroma-free hemoglobin solution in the late 1960s (23). Interest in stroma-free hemoglobin as a blood substitute arose primarily from its many advantages over crystalloid fluids. These include: 1) its existence as a naturally occuring protein; 2) its low viscosity; 3) its oxygen transporting capabilities; 4) the elimination of typing or crossmatching problems; 5) the absence of any significant allergenic problems; and 6) its ability to serve as an adequate plasma expander.

In addition, new manufacturing techniques allow the production of a true stroma-free hemoglobin solution which is devoid of the nephrotoxicity previously associated with stromal components (13,24). Further work has helped solve the additional problems of intravascular retention and oxygen offloading (10,11,16). Its use as a resuscitation fluid has been reported (9,15,19,24) and others have demonstrated increased survival with the use of these solutions at hematocrits of 5% or less (6,18,22). These authors all reported increased effectiveness of stroma-free hemoglobin solution over other crystalloid resuscitation fluids demonstrating that acid-base status, mitochondrial function, and myocardial function are preserved adequately if hemoglobin solution, rather than a non-hemoglobin containing solution, is used.

Although these studies have been valuable in establishing the "gross efficacy" of the solutions, they have not characterized oxygen transport completely in these animals, nor have they offered much information regarding the efficacy of the solutions in less anemic conditions. Furthermore, nothing is known about the ability of stroma-free hemoglobin solution to meet the increased oxygen demands associated with exercise.

This study was designed to compare the effects of stroma-free hemoglobin solution and 7% albumin solution, in conscious animals exchange-transfused to equal hematocrit levels and examined under conditions of rest and maximal exercise. Specifically, we tested the following hypothesis: since exercise performance is dependent on total oxygen transport and oxygen consumption, is performance maintained more successfully in exercising swine following hemodilution with stroma-free hemoglobin solution rather than with albumin.

METHODS

Animal Model

Fourteen swine (40-50 kg) of either sex were chronically instrumented in a manner similar to that previously reported (25). The surgical preparation was performed under anesthesia consisting of induction with ketamine (1 mg/kg, IM) and surital (20 mg/kg, IV), and maintenance with a combination of 0.5% halothane with oxygen and intravenous succinylcholine (400 mg/L at 7 ml/min). A left lateral thoracotomy was performed through the fourth intercostal space and the pericardium was opened. Left ventricular internal diameter ultrasonic dimension crystals were implanted as indicators of global heart mechanics. These internal diameter crystals were positioned by pulling one crystal with its wire through the left ventricular lateral wall with a large needle in the manner described by Bishop (3). The crystal remained in the left ventricular chamber against the septum while the lead wires continued out through the septum and right ventricle. The second crystal was placed on the endocardium of the lateral wall through the track created by the passage of the first crystal. Silastic catheters (.085" ID) were placed in the descending thoracic aorta (to monitor pressure, collect blood samples for blood gas and oxygen content analysis, and to serve as a port for the withdrawal of blood samples during microsphere injection), the pulmonary artery (to obtain a mixed venous sample for blood gas and oxygen content analysis), and the left atrium (to monitor pressure and to inject microspheres). An 18 mm (internal diameter) electromagnetic flow probe (Biotronix, Silver Springs, Maryland) was placed around the ascending aorta to measure cardiac output (CO) and ejection velocity.

All catheters and lead wires were brought out of the thoracic cavity via the fourth intercostal space and then run subdermally to the back where they

were externalized. A diagram of this instrumentation is presented in Figure 1.

Myocardial Function

Ventricular dimensions were measured using implanted ultrasonic crystals connected to a Schuessler and Associates Sonomicrometer (Sonotek, San Diego, Ca.). The ultrasonic signal was monitored with a Tectronic 465B oscilloscope (Beaverton, Ore.) and was recorded on an Elema-Schonander Minograf 81 ink jet recorder (Stockholm, Sweden).

Global left ventricular function was measured using the technique of Bishop (3). Changes in internal diameter during ventricular ejection (%ΔD) were defined as EDD-ESD x 100 divided by EDD; where EDD is end-diastolic diameter and ESD is end-systolic diameter. EDD was defined as the time coincident with the peak of the R wave of the EKG, and ESD as the time point of minimal chamber diameter.

Regional Blood Flow

Distribution of cardiac output was determined by injection of carbonized microspheres (15 $\pm$ 10μm, New England Nuclear, Boston, Mass.) in a manner previously reported (26). Regional blood flow was calculated by the method described by Domenech (8) allowing flows to be expressed in ml/min/g tissue (wet weight). Tissue blocks of approximately 5 grams were taken from the brain, epicardium, endocardium, kidney, spleen, intestine, stomach, and skeletal muscle regions. These samples were minced, dried, and counted in a Packard-Auto Gamma Spectrophotometer model 5912 equipped with a multichannel analyzer. A Hewlett-Packard 9825A programmable calculator was used to calculate radioactivity per gram of tissue. Analysis of the energy spectra was performed according to the matrix inversion method of Schosser (27). Standards, containing pure radionuclide, provided overlap matrix values. Unknown amounts of

the radionuclide in all samples were determined by solving a system of simultaneous linear equations. Validity checks were made by counting sealed radionuclide samples separately and then counting them together in a single sample. These validation procedures demonstrated an error of less than 3%.

Blood gas analysis (Instrumentation Laboratories 813, Lexington, Mass.) and oxygen content determinations (I.L. 282 and Lexington Instruments:Lex O_2 Con) were derived from blood samples taken from the arterial and venous catheters just prior to microsphere injection.

Total oxygen consumption was defined as arterial-venous oxygen content difference x cardiac output (ml/kg/min). Total oxygen transport (ml O_2/kg/min) was calculated from arterial oxygen content x cardiac output. Lactate determinations were done using the technique of Beutler (2). Microspheres detected in the lungs provided data for the percent of cardiac output shunted around the capillary beds (12).

Animal Protocol

Familiarization of the animals with treadmill exercise was carried out in the manner previously reported by this laboratory (32). Two weeks following surgical instrumentation a progressive continuous treadmill stress test was administered to each animal on two successive days. These data were needed in order to determine the maximum heart rate of each animal and to determine the normal exercise capacity of each animal. The animals had been adapted to treadmill running by progressive stress testing prior to surgery. The treadmill protocol consisted of the following two-minute stages of increasing work load: 2 mph/15% grade, 3.1 mph/5% grade, 3.1 mph/10% grade, 3.1 mpn/15% grade, and 3.1 mph/20%. Maximal oxygen consumption was determined when increased effort no longer elicited a further increase in heart rate or oxygen consumption. An electrically charged grid at the rear of the treadmill

discouraged halting before exhaustion. Previous studies from our laboratory haveshown that the pig can achieve and sustain maximal heart rate for several minutes while final measurements are made (31). It was anticipated that animals that received a blood exchange would have a greatly decreased work capacity. In two pilot studies with animals not included in this study we exchanged SFHS or albumin and attempted to repeat the progressive stress test. Neither animal was able to achieve more than the third workload stage (3.1 mph/10% grade). Furthermore, each animal stopped abruptly during the third stage, suggesting that the steady-state condition necessary for microsphere measurements could not be achieved. Therefore, the exercise program following exchange consisted of stage 1 and stage 2 workloads with stage 2 continuing until the animal was no longer able to run. Microspheres were injected at a heart rate previously determined as maximum, and maximum oxygen consumption was determined from the aortic flow probe and blood gas measurements before exercise termination. Maximal heart rates were similar between the control progressive test and the modified post-exchange progressive test. Exercise capacity was measured in total time to exhaustion (minutes) and in total work performed (kg/meters) calculated by meters/min x percent grade x kg (body weight) x minutes. Control pre-exchange exercise work performance of approximately 2000 kg/meter was similar to values obtained in other control exercise runs in our laboratory.

Experimental Design

Four conditions were studied in each animal. Control data for the resting state was collected with the animal standing quietly on the treadmill. These measurement procedures consisted of recording hemodynamic measurements, taking arterial and venous blood samples, and injecting a dose of tracer microspheres (approximately 5 x 10^6 spheres) into the left atrial

catheter. These procedures were repeated during maximal exercise conditions. Following a 30 minute recovery, at which time the normal resting conditions were re-established, each animal was exchanged to an average hematocrit level of 15% with either hemoglobin solution or albumin solution. Exchange time was 1.5 hours. Post-exchange control and maximal exercise measurements were made. The following measurements were obtained at each recording: cardiac output, arterial and left atrial pressure, heart rate, stroke volume, change in flow per unit time (dF/dt), and sonomicrometry-measured ventricular dimensions.

Data Analysis

Statistical analysis included the use of one-way analysis of variance with a Newman-Keuls multiple range test for between group and condition testing. Paired t-tests were performed within the groups for condition testing (exchange vs. control; exercise vs. rest).

Two animals died during exercise following albumin exchange with ventricular fibrillation and could not be included in this study. Therefore, six animals were used in both the stroma-free hemoglobin solution and in the albumin solution groups.

Preparation of the Solutions

Two solutions having similar oncotic and colloid pressures were prepared for these experiments. The albumin solution was prepared using serum bovine albumin from a commercial source (Cal Biochem, La Jolla, California). This bovine albumin was suspended in hemodialysis fluid to provide a final concentration of approximately 7 g/DL. The stroma-free hemoglobin solution was prepared using a modification of the technique described by Greenburg (10). This solution was prepared in order to provide a final concentration of approximately 7 g/DL of hemoglobin. A typical batch of our stroma-free hemoglobin

solution was characterized by a hemoglobin concentration of 6.1 g/DL, a methemoglobin concentration of 0.9%, normal serum electrolytes, an osmolarity of 32 mOsm, and a p50 of 14.9 TORR. All solutions were used within 90 days of manufacture (storage at 4°C) and periodic pyrogen testing and determination of metnemoglobin levels insured the use of a sterile product with methemoglobin level less than 3%. Although we did not directly measure the p50 of the post-exchange in vivo blood and SFHS mixture in these pigs, we have noted in previous studies (21) that the resultant in vivo p50 drops from a normal value of approximately 35 TORR to a reduced value of 27 TORR.

RESULTS

All results are shown in Tables 1-4 and Figures 3-5. An example of actual pressure dimension and flow tracings during rest and exercise is seen in Figure 2. Specific illustrations for coronary blood flow, oxygen consumption, and aortic pressure are shown in Figures 3, 4, and 5 respectively.

As can be seen in Table 1, control animals in the two groups had hematocrits of approximately 30%, which increased slightly with exercise. Following hemodilution with either albumin or stroma-free hemoglobin solution, resting hematocrits of 14% increased to 18% with exercise.

Albumin Exchanged Animals

Resting Condition: Arterial oxygen content and A-V O_2 difference was significantly lower than control following exchange-transfusion with albumin (Table 1). Oxygen consumption (Figure 4) was not compromised due to increased cardiac output, heart rate, and dF/dt (Table 2). Lactate production did not increase significantly (Table 1). An increase in coronary blood flow was detected (Figure 3), but there were no changes in cerebral or visceral organ blood flows (Table 3). Myocardial function, as measured by % D, was not compromised (Table 4).

Exercising Condition: Arterial oxygen content, arterial-venous oxygen content difference, oxygen consumption (Figure 4), total oxygen transport, and aortic pressure (Figure 5) were decreased significantly when compared to control exercise conditions (Tables 1,2). Despite a decrease in heart rate, cardiac output remained unchanged because stroke volume increased. Both lactate production and left atrial pressure showed increases compared to control exercise (Tables 1,2), while aortic pressure was significantly lower (Figure 5). The albumin exchange resulted in significant increases in both coronary and cerebral blood flow, as well as decreases in mean visceral organ flow (Table 3). Albumin-exchanged animals could only run for a period of time equal to approximately 50% of their control exercise time, but this decreased performance was not significantly different from the 60% performance achieved by the hemoglobin animals (Table 4).

Stroma-Free Hemoglobin Solution

Resting Condition: As noted following albumin exchange, stroma-free hemoglobin solution exchange-transfused animals had a significant drop in arterial oxygen content. This decrease was not of the magnitude found following albumin exchange. Despite this drop, no changes occurred in oxygen consumption, cardiac output, heart rate, lactate production (Tables 1,2), or organ blood flow (Table 3). As with the albumin-exchanged animals, myocardial function was not changed (Table 4).

Exercising Condition: During exercise, both arterial oxygen content and arterial-venous oxygen content differences were significantly lower than control exercise values (Table 1). Cardiac output remained similar to control levels (Table 3), but unlike the albumin animals, the stroma-free hemoglobin solution animals showed no changes in oxygen consumption, oxygen transport, lactate production, heart rate, or dF/dt (Table 2). In addition, these animals showed no detectable changes in organ blood flow (Table 3).

Microsphere Shunting

The results of the shunting measurements are shown in Table 3. With stroma-free hemoglobin solution and albumin solution, the shunt was increased significantly at rest and decreased significantly with exercise. Albumin and stroma-free hemoglobin solution animals appeared to affect microsphere shunting in a similar fashion.

DISCUSSION

The results from this experiment indicate that exercise performance was maintained more effectively by hemodilution with stroma-free hemoglobin solution than with 7% albumin. This is indicated by greater oxygen delivery, aortic pressure, exercise dF/dt, duration of exercise, and decreased lactate production in the stroma-free hemoglobin solution exchanged animals compared to the albumin-exchanged animals.

At rest following albumin exchange, oxygen consumption and oxygen transport remained close to control levels due to an increase in cardiac output. Because stroke volume remained constant, this increase in cardiac output was due to an increase in heart rate. This coincides with the results of other investigators (5,14) who reported that after a 50% reduction in hemoglobin concentration cardiac output varied inversely with hematocrit primarily due to decreased viscosity and peripheral vascular resistance. Increased cardiac output was not detected in animals exchanged with stroma-free hemoglobin solution which has a viscosity similar to the viscosity of albumin solution (7). Therefore, it may be that the increase in cardiac output is a combination of an autoregulatory mechanism in response to mild hypoxia as well as the decreased viscosity.

During exercise, albumin-exchanged animals had lower oxygen consumption when compared to pre-exchange exercise values. This indicates insufficient

oxygen to meet the higher oxygen demands of exercise. This was associated with a significant decrease in total oxygen transport due to the decreased transport of oxygen with albumin solution and may also be due to the increased peripheral blood shunting found in these animals. Evidence for this peripheral arterial-venous shunting is based on the decreased aortic pressure and lower peripheral resistance noted in the albumin-exchanged animals. Previous work from this laboratory (25) has shown that the decrease in absolute visceral blood flow is a sensitive indicator of exercise stress in the pig. This sensitivity is apparent when comparing the greater decrease in visceral blood flow in the albumin-exchanged exercised group compared to the other exercised groups.

If the ultimate limit of aerobic exercise is the limitation of oxygen transport or oxygen diffusion as has been suggested by Blomquist and Saltin (4), then the redistribution of blood flow away from the viscera during exercise may be enhanced further during hemodilution and exercise.

The degree of shunting based on microspheres trapped in the lung shown in these studies is low enough to be physiologically insignificant. It is of some interest that the degree of shunting at rest increases significantly with stroma-free hemoglobin solution and albumin solution, suggesting rheological alteration which might account for more arterial-venous shunting. However, since the point of maximal stress is during exercise and there is relatively low shunting during exercise under all conditions, it does not appear that either albumin or stroma-free hemoglobin solution results in enough shunting to have an adverse effect during exercise.

During exercise stress in the albumin-exchanged animals, heart rate did not increase cardiac output enough to compensate for the increased oxygen requirements. This may be explained by a failure to meet the necessary oxygen

requirements needed to elicit a maximal heart rate. Further support for this interpretation is provided by the fact that albumin-exchanged animals ran for an average of 1.6 minutes less than stroma-free hemoglobin solution-exchanged animals and approximately six minutes less than under control conditions.

Previous reports (14) have shown an increase in coronary blood flow with hemodilution. We also noted that coronary blood flow was greater following hemodilution with albumin during rest and exercise. Since there was no increase in coronary blood flow following stroma-free hemoglobin solution exchange, this suggests that these animals were not experiencing as high a level of hypoxia.

The constancy of $\%\Delta D$ and end-diastolic diameter suggests that despite decreased oxygen availability following exchange with albumin versus stroma-free hemoglobin solution, myocardial performance (determined by these indices) was not impaired. This apparent constancy of myocardial function may be deceiving, however, because we have little information regarding how long myocardial function could have been maintained under these conditions. In addition, albumin-exchanged exercised pigs experienced a significa decrease in aortic pressure and a marked increase in left atrial pressure during exercise. We have seen such changes often during initial stages of myocardial failure (28) and have attributed them to a coronary blood flow below the reserve capacity of the heart (31).

A recent investigation from our laboratory (17) on the effects of global hypoxia on myocardial function showed that indices of myocardial function such as % D and dP/dt increased with decreasing arterial PO_2. The point of critical oxygen delivery to the heart was characterized by a maximal dP/dt response and a maximal stroke volume. This critical point also results in maximal coronary vasodilation. With a further decrease in arterial oxyen content,

immediate "heart failure" is evident, as shown by a rapid decrease in dP/dt, stroke volume, $\%\Delta D$, arterial blood pressure, and a net production of myocardial lactate. This rapid decompensation of the heart may not be evident in an exercising animal since intense exercise is probably impossible at such low oxygen tensions. However, in this present study we experienced two episodes of sudden heart failure characterized by ventricular fibrillation in exercising animals following albumin exchange. These animals had no previous arrhythmias while exercising, but showed a sudden rapid decrease in blood pressure, dF/dt, and % D just before fibrillation. The data from these two animals were not used in this study since final blood flow studies were not obtained.

Additional support for the advantage of stroma-free hemoglobin solution over albumin is provided in the overall exercise performances. Although none of the exchanged animals in either group met control levels, stroma-free hemoglobin solution animals were able to run to a 60% capacity, while albumin animals were capable of reaching only 50% capacity. This 10% difference did not reach statistical significant and would not initially appear to be important. However, this 10% difference in measurable exercise endurance was accompanied by a qualitative difference in the manner in which the two groups tolerated their maximum exercise levels. The stroma-free hemoglobin solution-exchanged animals were almost always capable of continuing to exercise at their maximum heart rate, while the albumin animals had difficulty sustaining their maximum exercise. This difference in exercise performance was more dramatic in similar exercise studies utilizing a similar albumin and 50% stroma-free hemoglobin solution exchange in exercising nonthoracotomized dogs (20).

Lactate production following albumin exchange and exercise increased significantly even though total exercise time was slightly less than with stroma-free hemoglobin solution. Cain reported that increased lactate can be the result of "hypoxemia produced by autoexchange anemia" (5). Although we did not find increased lactate at rest following exchange, we did find increased lactate during exercise. Again, we are provided with evidence that albumin animals were experiencing a greater oxygen deficiency which ultimately led to a lower exercise capacity.

Although overall exercise performance was lower than control levels following stroma-free hemoglobin solution exchange, this study suggests that animals hemodiluted with such a solution have higher exercise capacity than animals hemodiluted with albumin solution.

An issue not directly addressed in this study is the potential toxicity of the solutions. The major problem of tissue damage from red cell stroma has generally been solved, however some recently published articles have documented significant hemodynamic and hematological toxicity not associated with the stromal components (29,30). Some reported abnormalities such as minor coagulation defects would not be detected in our study. However, the major hemodynamic problems such as significant arrhythmias, hypoxia, and death would have been detected. These were absent in our study because the hemoglobin solution-exchanged animals had improved hemodynamics and an improved response to exercise. These problems were reported in rabbits, and in view of the lack of any toxicity in our pig model or in our simpler canine model (20), it would seem reasonable to conclude that it is possible to manufacture an effective non-toxic solution. The animal model used to evaluate that solution is of crucial importance.

While the art and science of manufacturing stroma-free hemoglobin solution has not reached a level sufficient to provide a product that can function as a complete red cell substitute, we feel this study demonstrates an improvement in various physiologic parameters even when an imperfect solution is used. Furthermore, we were impressed that those beneficial effects were apparent at moderate levels of hemodilution, although the imposition of an exercise stress was essential to elucidate some of these differences.

REFERENCES

1. Amberson, W.R. On the use of Ringer-Locke solutions containing hemoglobin as a substitute for normal blood in mammals. J. Cell. Comp. Physiol. 5:359-382, 1934.

2. Beutler, E. Red Cell Metabolism: A Manual of Biochemical Methods. New York, Grune & Stratton, p. 108, 1971.

3. Bishop, V.B., L.D. Horwitz, H.L. Stone, H.F. Stegal, and E.J. Engelker. Left ventricular internal diameter and cardiac function in conscious dogs. J. Appl. Physiol. 5:619-623, 1969.

4. Blomquist, C.G. and B. Saltin. Cardiovascular adaptations to physical training. Ann. Rev. Physiol. 45:169-189, 1983.

5. Cain, S.M. Appearance of excess lactate in anesthetized dogs during anemic and hypoxic hypoxia. Am. J. Physiol. 209:604-610, 1965.

6. DeVenuto, F., W.Y. Moores, A.I. Zegna and T. Zuck. Total and partial blood exchange in the rat with hemoglobin prepared by crystallization. Transfusion 17:655-662, 1977.

7. DeVenuto, F. Viscosity of human blood hemodiluted with crystalline hemoglobin solution. Transfusion 21:752, 1981.

8. Domenech, G.J., J.I.E. Hoffman, M.I.M. Noble, K.B. Saunders, J.R. Henson and S. Subijanto. Total and regional coronary blood flow measured by radioactive microspheres in conscious and anesthetized dogs. Circ. Res. 25:581-596, 1969.

9. Elia, C., H.S. Steinberg, A.G. Greenburg and G.W. Peskin. Stroma-free hemoglobin in the resuscitation of hemorrhagic shock. Surg. Forum 25:201-204, 1974.

10. Greenburg A.G., M. Schooley and G.W. Peskin. Improved retention of stroma-free hemoglobin solution by chemical modification. J. Trauma 17:501-504, 1977.

11. Greenburg, A.G., G.W. Peskin, D.B. Hoyt and W.Y. Moores. Is it necessary to improve the intravascular retention of hemoglobin solutions? Crit. Care Med. 10:266-269, 1982.

12. Heymann, M.A., B.D. Payne, J.I.E. Hoffman and A.M. Rudolph. Blood flow measurements with radionuclide-labeled particles. Prog. Cardiovasc. Dis. 30:55-79, 1977.

13. Jaenike, J.R. and E.F. Schneeberger. The renal lesion associated with hemoglobinemia. I. First structured characteristics in the rat. J. Exp. Med. 123:537-545, 1966.

14. Jan, K.M. and S. Chien. Effect of hematocrit variations on coronary hemodynamics and oxygen utilization. Am. J. Physiol. 233:H106-H113, 1977.

15. Jesch, F., B. Endrich, R. Pfeiffer and K. Messmer. Resuscitation from acute blood loss with use of stroma-free hemoglobin solution. Emerg. Surg. Res. 8:168-169, 1976.

16. Kothe, N., Eichentopf, B. and Bonhard K. Characterization of a modified stroma-free hemoglobin. Surg. Gynecol. Obstet. 161:563-569, 1985.

17. Liu, Y., F.C. White, D. Willford, E. Hill and C.M. Bloor. Protection by cold of hypoxia induced ultrastructural damage in the myocardium. Lab. Invest. 50:35A, 1984.

18. Moores, W.Y., F. DeVenuto, W.H. Heydorn, R.B. Weiskopf, B.S. Baysinger, A.G. Greenburg and J.R. Utley. Extending the limits of hemodilution on cardiopulmonary bypass using stroma-free hemoglobin solution. J. Thorac. Cardiovasc. Surg. 81:155-162, 1981.

19. Moores W.Y., F. DeVenuto, W.H. Heydorn, A.G. Greenburg and J.R. Utley. Effectiveness of stroma-free hemoglobin solution as seen in a right heart bypass swine model. Crit. Care Med. 10:279-282, 1982.

20. Moores, W.Y., K. Gallagher, R.E. Mack, J. Lindsay, R. Schuessler, S. Kemper and J. Ross Jr. Chronic exercise response of the dog following hemodilution induced with albumin and stroma-free hemoglobin solutions. (manuscript submitted 1987).

21. Moores, W.Y., D. Sansonetti, A.G. Greenburg, R.E. Mack, D.C. Willford, R. Schuessler. Hemodilution on cardiopulmonary bypass: Efficacy of stroma free hemoglobin solution as a hemodiluting prime during cardiopulmonary bypass. In Proceedings of the Symposium of Thirty Years of Extracorporeal Circulation. S. Hagl, et al., editors. Munich, Germany, pp. 183-197, 1984.

22. Moss, G.S., R. DeWoskin, A.L. Rosen, H. Levine and C.K. Palani. Transport of oxygen and carbon dioxide by hemoglobin-saline solution in the red cell free primate. Surg. Gynecol. Obstet. 142:357-362, 1976.

23. Rabiner, S.F. Evaluation of a stroma-free hemoglobin solution for use as a plasma expander. J. Exp. Med. 126:1127-1142, 1967.

24. Reliham, M. and M.S. Litwin. Clearance rate and renal effects of stroma-free hemoglobin in acidotic dogs. Surg. Gynecol. Obstet. 137:73-79, 1973.

25. Sanders, M., F.C. White and C.M. Bloor. Cardiovascular responses of dogs and pigs exposed to similar physiologic stress. Comp. Biochem. Physiol. 58:365-370, 1979.

26. Savage, R.M., B.S. Guth, F.C. White, A.D. Haan and C.M. Bloor. Correlation of regional myocardial blood flow and function with myocardial infarct size during acute myocardial ischemia in the conscious pig. Circulation 64:699-707, 1981.

27. Schosser, R., E. Arforske and K. Messmer. Mic-II-a program for the determination of cardiac output, arterio-venous shunt, and regional blood flow using the radiomicrosphere method. Comp. Programs Biomed. 9:19-38, 1979.

28. Theroux, P., J. Ross, Jr., D. Franklin, J.W. Covell, C.M. Bloor and S. Sagayama. Regional myocardial function and dimension early and late after myocardial infarction. Circ. Res. 40:158-165, 1977.

29. White, C.T., A.J. Murray, D.J. Smith, J.R. Greene and R.B. Bolin. Synergistic toxicity of endotoxin and hemoglobin. J. Lab. Clin. Med. 108:132-137, 1986.

30. White, C.T., A.J. Murray, J.R. Greene, D.J. Smith, F. Medina, G.T. Makovec, E.J. Martin and R.B. Bolin. Toxicity of human hemoglobin solution infused into rabbits. J. Lab. Clin. Med. 108:121-131, 1986.

31. White, F.C., M. Sanders and C.M. Bloor. Coronary reserve at maximal heart rate in the exercising swine. J. Cardiac Res. 1:31-40, 1981.

32. White, F.C., M.B. McKirnan, E.F. Breisch, B.D. Guth, Y.-M. Liu and C.M. Bloor. Adaptation of the left ventricle to exercise-induced hypertrophy. J. Appl. Physiol. 62(3):1097-1110, 1987.

FIGURE LEGENDS

Figure 1 Schematic diagram of instrumented swine heart illustrating aortic flow probe, catheter, and crystal placement.

Figure 2 Examples of actual pressure, dimension, flow, and the first derivative of flow (dF/dt) tracings.

Figure 3 Coronary blood flow measured in ml/min/100 grams.

o = Albumin control △ = Albumin exchange o = SFHS control

▲ = SFHS exchange * = $p<.025$, exchange vs. others

* = $p<.025$, exchange vs. control.

Values displayed are mean ± S.E.M.

Figure 4 Oxygen consumption measured in ml/min/kg.

o = Albumin control △ = Albumin exchange o = SFHS control

▲ = SFHS control † = $p<.01$, exchange vs. control

Values displayed are mean ± S.E.M.

Figure 5 Aortic pressure measured in mm/Hg.

o = Albumin control △ = Albumin exchange o = SFHS control

▲ = SFHS exchange * = $p<.05$, exchange vs. others

Values displayed are mean ± S.E.M.

TABLE 1. OXYGEN DYNAMIC MEASUREMENTS COMPARING ALBUMIN SOLUTION VS. STROMA-FREE HEMOGLOBIN SOLUTION

	ALBUMIN				STROMA-FREE HEMOGLOBIN SOLUTION			
	CONTROL		EXCHANGE		CONTROL		EXCHANGE	
	REST	EXERCISE	REST	EXERCISE	REST	EXERCISE	REST	EXERCISE
Hematocrit (%)	31 ± 4	36 ± 3	14 ± 3	18 ± 4	30 ± 4	33 ± 3	14 ± 1†	18 ± 1†
Arterial Oxygen Content (ml/dl)	12.7 ± 2.5	13.8 ± 3.2	6.2 ± 1.8*	6.6 ± 1.1*	14.0 ± 2.7	15.6 ± 2.4	9.8 ± 1.1†	11.0 ± 1.7†
Venous Oxygen Content	6.6 ± 1.8	3.1 ± 0.5	2.7 ± 0.6	1.1 ± 0.3	6.3 ± 1.4	3.4 ± 0.5	3.9 ± 0.3	1.7 ± 0.3
Arterial-Venous Oxygen Content Difference (ml/dl)	6.1 ± 2.0	10.7 ± 1.3	3.5 ± 1.9*	5.5 ± 1.5*	7.7 ± 2.5	12.2 ± 1.7	5.9 ± 0.6	9.3 ± 1.5†
Oxygen Consumption (ml/kg/min)	6.8 ± 1.9	27.4 ± 8.3	6.4 ± 2.4	16.5 ± 5.4†	7.4 ± 2.3	25.7 ± 5.2	5.9 ± 1.2	23.9 ± 6.3
Oxygen Transport (ml/kg/min)	14.5 ± 3.9	35.1 ± 11.2	11.3 ± 4.6	17.3 ± 4.3*	13.8 ± 3.9	33.2 ± 8.9	9.7 ± 1.9	28.1 ± 7.4
Lactate (mM/l)	0 ± 0	17 ± 2.2	2.8 ± 2.2	23.5 ± 4.3†	0.7 ± 0.9	16.0 ± 3.0	2.0 ± 3.0	20.4 ± 4.5
Mixed Venous PO_2 (mm Hg)	37 ± 2	20 ± 6	33 ± 6	33 ± 9	32 ± 6	21 ± 4	26 ± 6	28 ± 15

Values are mean ± S.E.M.
* = $p<.05$, Albumin exchange vs. others (Anova, 1-way)
† = $p<.05$, Exchange vs. control (paired t test)

TABLE 2. HEMODYNAMIC MEASUREMENTS COMPARING ALBUMIN SOLUTION WITH STROMA-FREE HEMOGLOBIN SOLUTION

	ALBUMIN				STROMA-FREE HEMOGLOBIN SOLUTION			
	CONTROL		EXCHANGE		CONTROL		EXCHANGE	
	REST	EXERCISE	REST	EXERCISE	REST	EXERCISE	REST	EXERCISE
Heart Rate	113 ± 10	257 ± 19	151 ± 21*	231 ± 20*	123 ± 22	262 ± 20	108 ± 13	258 ± 14
Cardiac Output (ml/min/kg)	116 ± 31	260 ± 86	179 ± 45*	264 ± 57	98 ± 19	211 ± 36	99 ± 13	251 ± 37
Stroke Volume (ml)	41 ± 7	42 ± 13	48 ± 12	49 ± 13	34 ± 10	33 ± 6	39 ± 10	40 ± 7
Left Atrial Pressure (mm Hg)	6.4 ± 3.8	12.9 ± 3.7	8.4 ± 4.7	16.3 ± 6.0	5.5 ± 4.9	11.0 ± 7.0	9.0 ± 7.6	13.0 ± 89
Aortic Pressure (mm Hg)	114 ± 9	131 ± 12	97 ± 19	107* ± 12	105 ± 15	127 ± 13	123 ± 15	150 ± 22*
df/dt	7.8 ± 1.7	17.6 ± 5.2	11.9 ± 3.3*	13.4 ± 1.8	7.2 ± 1.2	16.0 ± 2.9	7.4 ± 1.6	15.7 ± 3.6

Values are mean ± S.E.M.
* = $p<.05$, Exchange vs. others (Anova, 1-way)
† = $p<.05$, Exchange vs. control (paired t test)

TABLE 3. BLOOD FLOW MEASUREMENTS (ml/min/100 g tissue) COMPARING ALBUMIN SOLUTION VS. STROMA-FREE HEMOGLOBIN SOLUTION

	ALBUMIN				STROMA-FREE HEMOGLOBIN SOLUTION			
	CONTROL		EXCHANGE		CONTROL		EXCHANGE	
	REST	EXERCISE	REST	EXERCISE	REST	EXERCISE	REST	EXERCISE
Coronary	100 ± 29	318 ± 79	310 ± 167*	652 ± 182†	106 ± 30	424 ± 139	178 ± 59	568 ± 109
Brain	49 ± 10	49 ± 12	55 ± 13	97 ± 25*	42 ± 11	48 ± 13	51 ± 11	69 ± 23
Skeletal Muscle	9 ± 9	42 ± 35	9 ± 14	76 ± 84	4 ± 3	46 ± 24	4 ± 3	80 ± 33
Kidney	244 ± 90	59 ± 58	273 ± 107	5 ± 5	161 ± 97	55 ± 57	139 ± 102	70 ± 103
Intestine	40 ± 27	11 ± 9	44 ± 27	2 ± 2	34 ± 34	12 ± 15	37 ± 36	14 ± 18
Stomach	16 ± 9	10 ± 11	18 ± 13	0.3 ± 0.3	17 ± 14	12 ± 11	20 ± 11	6 ± 10
Liver	31 ± 30	19 ± 17	34 ± 24	9 ± 15	26 ± 18	17 ± 11	18 ± 7	16 ± 22
Spleen	332 ± 137	64 ± 73	255 ± 273	3 ± 6	222 ± 90	48 ± 73	139 ± 82	
Endo/Epi	1.2 ± 0.2	1.1 ± 11	1.1 ± 0.3	0.8 ± 0.2	1.0 ± 0.3	0.9 ± 0.2	1.2 ± 0.2	1.1 ± 0.2
Lung (% shunt)	4.7 ± .96	.88 ± .32§	9.3 ± 2.7†	1.95 ± 0.5§	4.2 ± 1.0	1.3 ± .4§	7.2 ± .3	1.1 ± 0.5†

Values are mean ± S.E.M.
Lung measurements are represented by % peripheral blood shunting
* = $p<.025$, Exchange vs. others (Anova, 1-way)
† = $p<.025$, Exchange vs. control (paired t test)
§ = $p<.05$, Exercise vs. rest (paired t test)

TABLE 4. EXERCISE PERFORMANCE AND MYOCARDIAL FUNCTION MEASUREMENTS COMPARING ALBUMIN VS. STROMA-FREE HEMOGLOBIN SOLUTION

	ALBUMIN				STROMA-FREE HEMOGLOBIN SOLUTION			
	CONTROL		EXCHANGE		CONTROL		EXCHANGE	
	REST	EXERCISE	REST	EXERCISE	REST	EXERCISE	REST	EXERCISE
Total work (kg/meter)		1942 ± 535		981 ± 297†		2052 ± 278		1245 ± 294†
Time to exhaustion (min)		11.4 ± 2.2		5.7 ± 1.2†		12.1 ± 2.2		7.3 ± 1.8†
% Diameter Shortening	26 ± 11	27 ± 12	27 ± 10	25 ± 8.5	29 ± 6	32 ± 9	25 ± 1	35 ± 7
End-diastolic Diameter (mm)	22 ± 3	22 ± 4	22 ± 3	22 ± 3	21 ± 2	20 ± 3	22 ± 0.4	19.5 ± 2

Values are mean ± S.E.M.
† = $p<.005$, Exchange vs. control (paired t test)

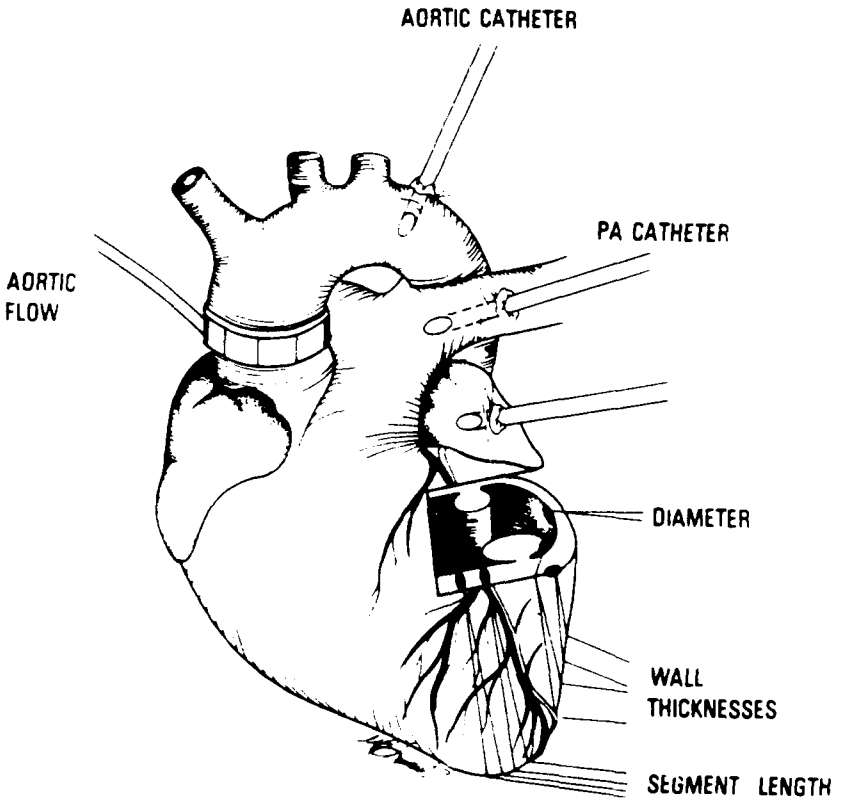
AORTIC CATHETER
PA CATHETER
AORTIC
FLOW
DIAMETER
WALL
THICKNESSES
SEGMENT LENGTH

FIG. 1

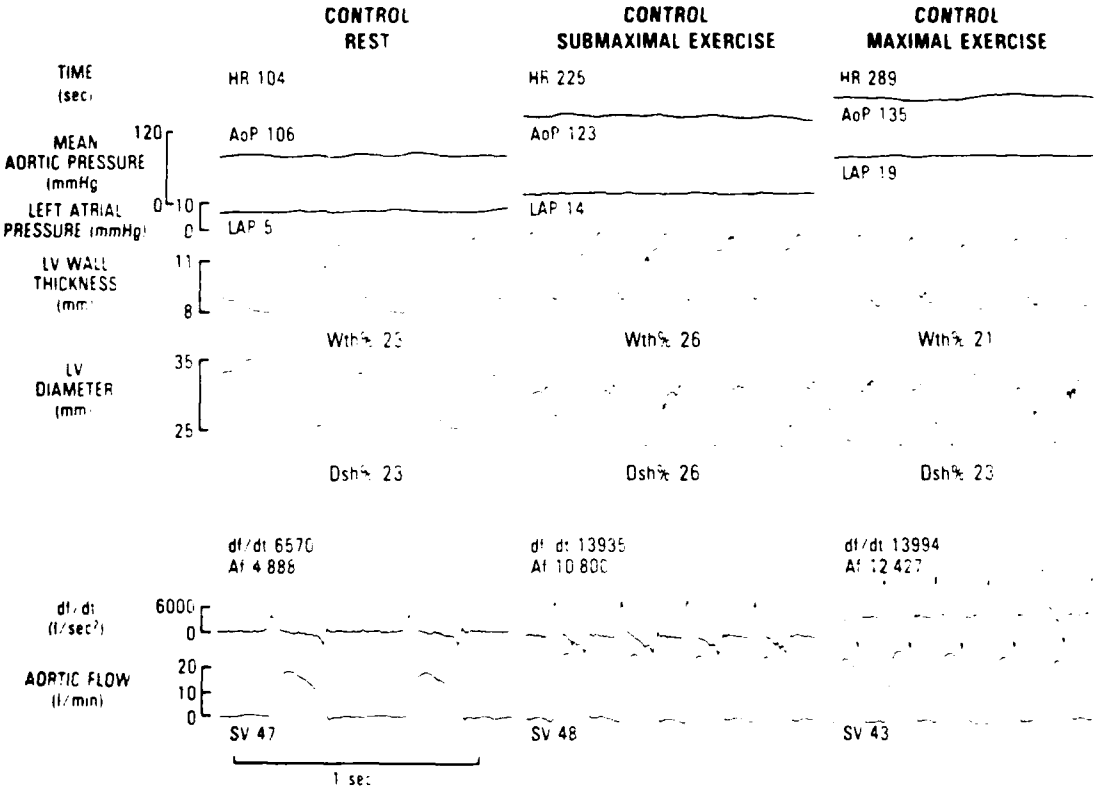
CONTROL
REST
CONTROL
SUBMAXIMAL EXERCISE
CONTROL
MAXIMAL EXERCISE
TIME
(sec)
HR 104
HR 225
HR 289
AoP 135
MEAN
AORTIC PRESSURE
(mmHg)
120
AoP 106
AoP 123
LAP 19
LEFT ATRIAL
PRESSURE (mmHg)
0
10
0
LAP 5
LAP 14
LV WALL
THICKNESS
(mm)
11
8
Wth% 23
Wth% 26
Wth% 21
LV
DIAMETER
(mm)
35
25
Dsh% 23
Dsh% 26
Dsh% 23
dP/dt 6570
At 4 888
dP/dt 13935
At 10 800
dP/dt 13994
At 12 427
dP/dt
(P/sec²)
6000
0
AORTIC FLOW
(l/min)
20
10
0
SV 47
SV 48
SV 43
1 sec

FIG. 2

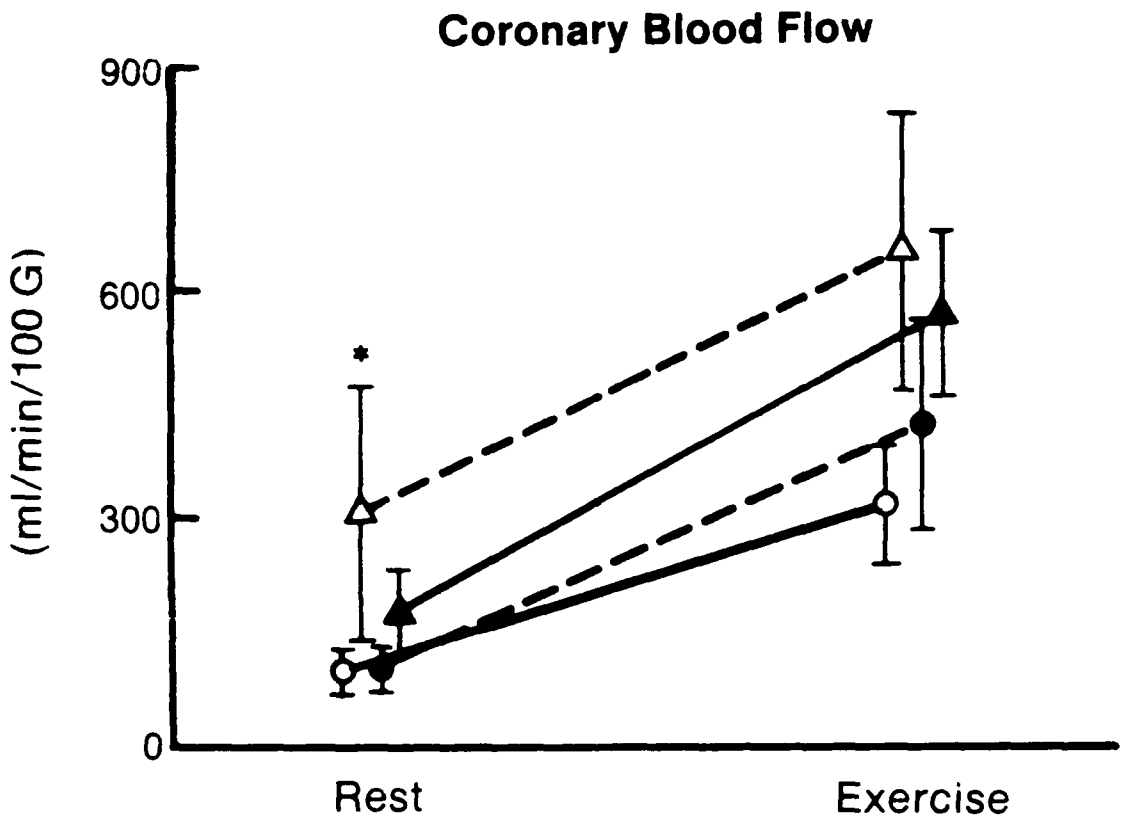

FIG. 3

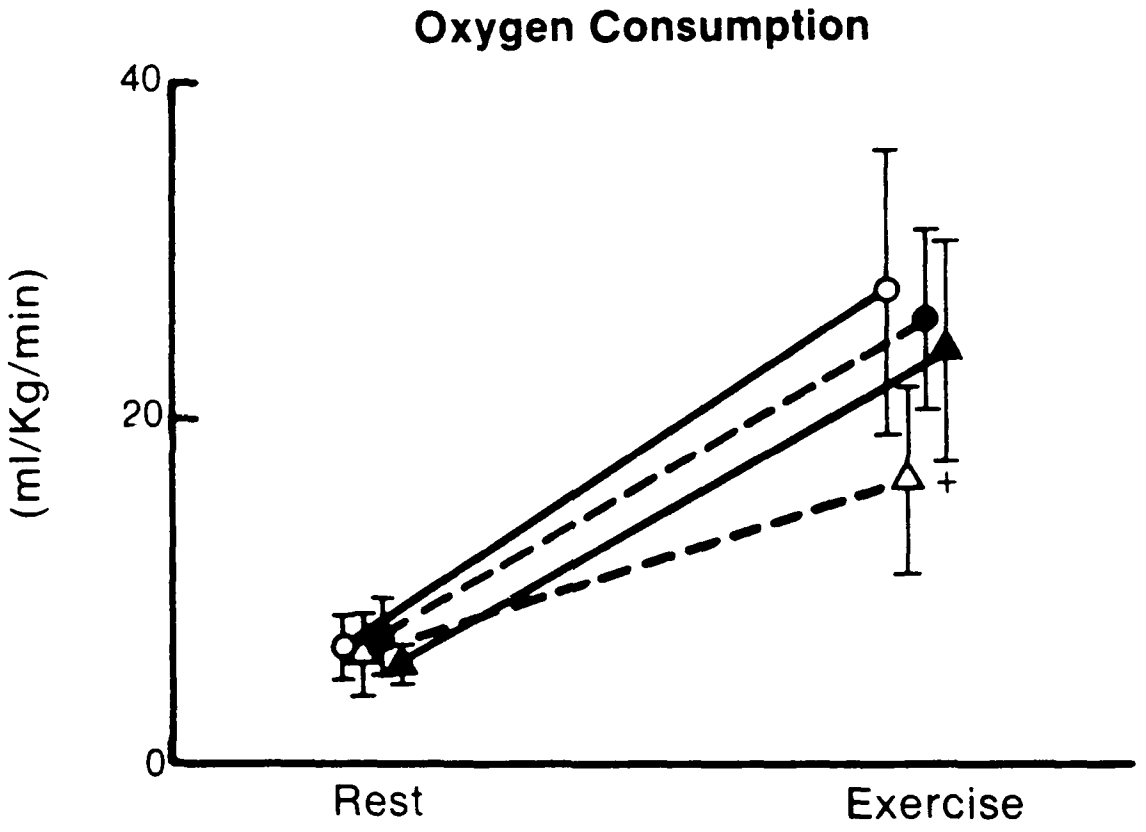

FIG. 4

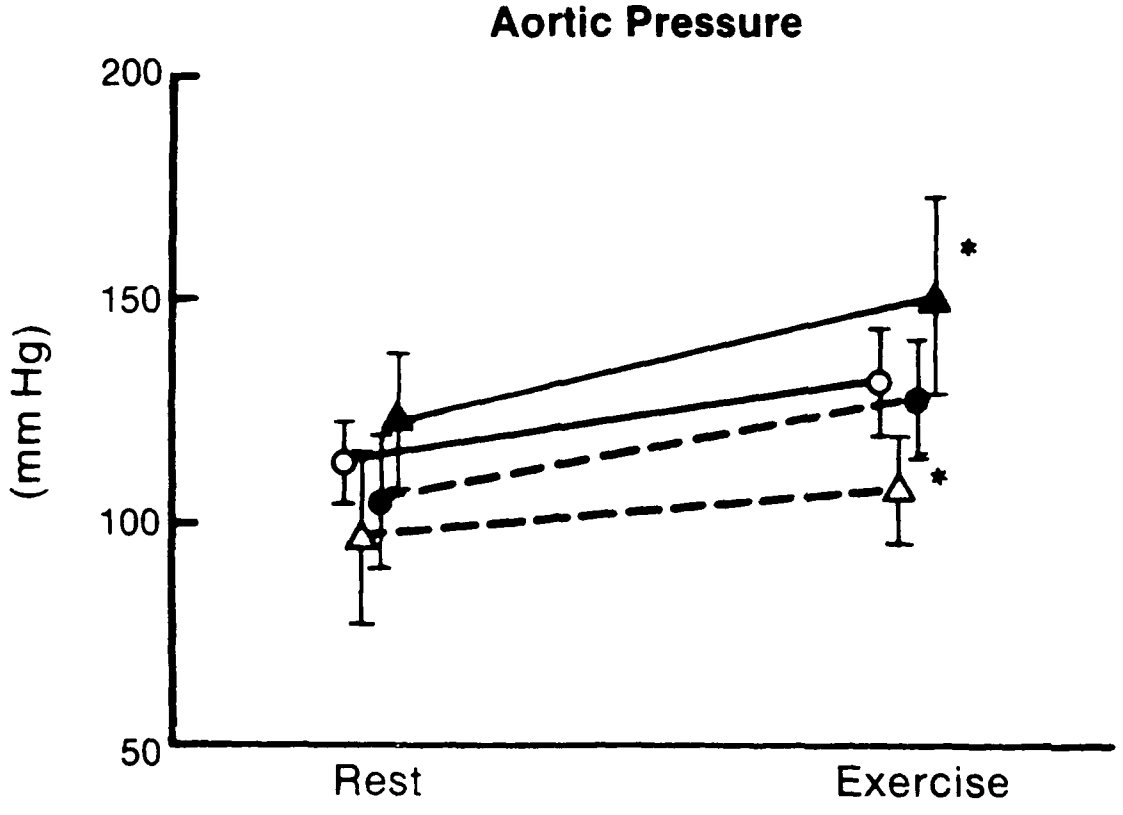

FIG. 5

(5)

CHRONIC EXERCISE RESPONSE OF THE DOG FOLLOWING HEMODILUTION INDUCED WITH ALBUMIN AND STROMA-FREE HEMOGLOBIN SOLUTIONS

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The opinions or assertions contained herein are the private views of the authors and are not to be construed as official or reflecting the views of the Department of the Army or the Department of Defense (AR 360-5)

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In conducting the research described in this report, the investigation adhered to the "Guide for the Care and Use of Laboratory Animals," as promulgated by the committee on revision of the "Guide for Laboratory Animal Facilities and Care," Institute of Laboratory Animal Resources, National Research Council

Running Head: Hemoglobin solutions in exercising dogs

ABSTRACT

The ability of stroma-free hemoglobin solutions to support a hemodiluted animal's ability to exercise following exchange transfusion was evaluated in 20 splenectomized mongrel dogs. Control measurements included hematocrit, heart rate, total exercise time, arterial and venous oxygen content and venous lactate levels during rest, exercise, and recovery. Five dogs comprise each group which was 50% exchange-transfused while awake with either: (1) a modified stroma-free hemoglobin solution (P_{50}= 22 TORR), (2) unmodified stroma-free hemoglobin solution (P_{50}= 11 TORR), (3) 7% bovine serum albumin, or (4) their own blood. Measurements were made immediately following transfusion, and at 24 hours, 48 hours, and seven days post-transfusion. Animals exchanged with either of the hemoglobin solutions were initially capable of exercising at a higher rate than the albumin-exchanged animals ($p < .05$). However, by the 48-hour and seven-day measurements, all animals had returned to control values. The higher P_{50} solution did not provide any major, statistically significant advantage over the unmodified solution.

KEY TERMS: Exercise oxygen supply
Artificial blood
Hemoglobins

INTRODUCTION

Among the experimental resuscitation fluids currently being evaluated, stroma-free hemoglobin solution (SFHS) appears to be an advantageous solution based primarily upon its ability to transport oxygen (1-3). In addition, it exists as a naturally occurring protein which can be transfused without any known allergic or cross-matching problems (4-5). In the lyophylized state, SFHS has a relatively long shelf life and has been reported previously as an "ideal" plasma expander (6-8). In addition, due to its low viscosity (8) SFHS potentially may be an excellent candidate for initiating hemodilution during cardiopulmonary bypass (9).

Despite these advantages, there are problems associated with the use of SFHS which need further investigation. One problem is the typically low P_{50} value (11-13 TORR) of hemoglobin solution, resulting in a leftward shift in the oxyhemoglobin dissociation curve with increased oxygen affinity (10). A second major problem is the relatively short biological retention time (11) of hemoglobin solution. In addition, although the kidney-damaging characteristics of the earlier solutions have been resolved with removal of red blood cell stroma (6,13,14), recent published reports (15,16) as well as unpublished communications from various laboratories using SFHS have raised the question of non-stroma related toxicity in the form of deleterious procoagulant, cardiovascular, and organ damaging effects. These toxic reactions have made the solution virtually unusable for many of these investigators. Initial investigations into the causes of these toxic reactions indicate that some animal models (e.g., rabbit) may be inappropriate, and that special care must be exercised to insure a high level of consistent quality control in the production of the solution as well as insuring that the final solution is chemically balanced and free of endotoxin and pyrogens. Since we did not experience any

of these non-stromal associated problems with our particular solutions, we have not addressed this toxicity issue in our studies. We have concentrated primarily on evaluating the effects of high oxygen hemoglobin affinity and shortened retention time in solutions used to effect hemodilution in a 50% awake exchange animal model.

Two of these problems of concern in this study have been addressed by Greenbury and associates reporting that "permatization" of SFHS with pyridoxal 5'-phostphate (PLP) not only improved P_{50} values without impairing oxygen carrying capacity, but also improved intravascular retention time over unmodified versions by 50% (12). More recent work from Europe by Kothe and associates (17) has resulted in further solution improvements utilizing intermolecular cross-linking as well as pyridoxalization. Despite these apparent advances in the composition of the solutions, there are few, if any, studies comparing these solutions under induced stress conditions (i.e., exercise) designed to evaluate the ability of these solutions to support a function known to require an increased oxygen consumption.

This study was designed therefore to compare the effects of three different hemodiluting fluids: 1) modified SFHS, 2) unmodified SFHS, and 3) 7% albumin solution in nonanesthetized dogs exchanged to equal hematocrit levels and examined under conditions of rest, exercise, and recovery. We specifically wanted to determine if the greater oxygen-carrying capacity of either SFHS provides any significant advantage in terms of supporting exercise capacity at a reduced circulating hematocrit.

METHODS

Twenty mongrel dogs (30-45 kg) of either sex which had been trained previously to run on a treadmill were used in this study. Following anesthesia with sodium pentobarbitol (25 mg/kg), arterial and venous catheters were

placed in the carotid and jugular veins for sampling purposes and the spleens were removed. The animals were allowed to recover for two weeks before experimentation began. At this time, control measurements were collected during rest, exercise, and recovery periods. These measurements included EKG-evaluated heart rate and arterial and venous blood samples for blood gas determination, hematocrit levels, arterial and venous oxygen content levels, and venous lactate levels. All exercise data and blood samples were collected after the dogs had completed four minutes of exercise including two minutes at 2 mph and two minutes at 4 mph. All recovery data was collected ten minutes post-exercise.

Following these control measurements, four groups of five animals each were exchange-transfused with either their own blood (Sham exchange), 7% albumin, modified SFHS, or unmodified SFHS to an average hematocrit level of 16% (except for the Sham-exchanged animals whose hematocrit level remained the same). Transfusion volume was approximately 1.5-2 liters per dog and required approximately one hour to complete. All Sham dogs had two liters of their own blood withdrawn and reinfused in a manner identical to the animals being exchanged with an exogenous solution.

Following transfusion, animals were again placed on the treadmill and the measurement sequence previously outlined was repeated. Additional measurements consisting of hematocrit, arterial oxygen content, total run time, and heart rate during rest, exercise, and recovery were collected 24 hours, 48 hours, and seven days post-transfusion.

Unmodified SFHS was prepared in our laboratory in a manner previously reported (6,10,13,14). The average P_{50} of this solution was 11 TORR. Modified SFHS was prepared in our laboratory according to Greenburg's method (6). The P_{50} of this solution was approximately twice that of unmodified SFHS or 22

TORR. Seven percent albumin solution was prepared using bovine serum albumin Fraction Five (Calbiochem, La Jolla, Ca.). All P_{50} measurements were accomplished using a Radiometer Dissociation Curve Analyser (model DCA1). Blood gas results were obtained using Instrumentation Laboratories (I.L.), model 813 Blood Gas Analyzer (Lexington, Ma.). Arterial and venous oxygen content results were obtained using an I.L. 282 Co-oxymeter. Venous lactate levels were determined according to the technique of Beutler (18). All exercise runs were performed in a standard clinical treadmill starting at 0 grade and progressing in speed and grade. A complete exercise run consisted of completing two minute runs at 2, 4, and 6 miles per hour at 0° grade followed by four minute runs at 8 miles per hour at a 0%, 3%, and 5% grade. Total run time was measured in minutes with a completed exercise run taking 18 minutes.

Statistical analysis consisted of using Analysis of Variance, 1 way, followed by a Newman-Keuls Multiple Range Test.

RESULTS

All animals receiving either a Sham exchange or either of the hemoglobin solutions ran at levels approaching control, as opposed to albumin-exchanged animals which were significantly limited ($p > 0.05$) in their exercise capabilities (Fig. 1). It should be noted that only two of five albumin animals were capable of exercising following exchange and therefore a zero for total run time was the average for each of the three animals that did not run.

After 24 hours, unmodified SFHS animals experienced a significant drop ($p<0.05$) in total run time compared to animals receiving modified SFHS or animals receiving a Sham exchange. All albumin animals were capable of exercising at the 24-hour testing and increased their average running time from 2.3 to 9.7 minutes. Despite this increase, albumin-exchanged and unmodified SFHS animals had a total exercise time statistically lower than the modified SFHS

group. After 48 hours, the exercise performance of the unmodified SFHS animals continued to be depressed while the albumin-exchanged animals continued to increase and met the level of the unmodified SFHS groups. At this point exercise performance between the two groups was virtually the same. After seven days all animals were able to recover sufficiently to approach control levels.

Figure 2 shows that all animals were exchanged to similar hematocrit levels. By seven days post-transfusion all but the albumin group had returned very close to control levels.

Heart rate results during rest, exercise, and recovery over the seven-day observation period are shown in Figures 3a, 3b, and 3c. Immediately following exchange and while still at rest, albumin-exchanged animals had significantly higher heart rates when compared to all other animals ($p<.05$). Because only two albumin-exchanged animals were capable initially of exercising following exchange, heart rate results for both exercise and recovery were limited to two samples for this group at the immediate post-exchange time period. Although the average heart rates of these two albumin-exchanged animals were higher during exercise and recovery than that of the Sham group or either hemoglobin group, the lack of adequate sample size prevents us from making any statistical statement on the significance of this finding. However, none of the modified or unmodified SFHS animals showed any significant changes in resting heart rate throughout the entire experiment. After 24 hours, the heart rates of the albumin-exchanged animals were back to control levels and remained at those levels to the study's conclusion.

Arterial oxygen content results are seen in Figure 4. Immediately following exchange, all three non-Sham groups experienced significant drops in oxygen content when compared to the Sham-exchanged animals ($p < 0.01$). In

addition, the oxygen content of the albumin-exchanged animals was significantly lower than either of the two hemoglobin groups ($p < 0.05$).

Throughout the 48-hour period there was a slight decrease in arterial oxygen content for both hemoglobin groups, while the oxygen content of the albumin-exchanged animals increased slightly making the three groups indistinguishable. However, by day seven both SFHS groups had increased their oxygen content levels to control values while the albumin-exchanged group, although continuing to increase slowly, had values significantly lower than all three other groups ($p < .05$).

Arterial-venous oxygen content values obtained before and immediately after exchange are presented in Table 1 during rest, exercise, and recovery conditions. No significant changes were detected during resting conditions, but during exercise and recovery the two albumin-exchanged animals capable of exercise differed from the other three groups, having lower mean arterial-venous oxygen content differences. Since cardiac output was not measured in our animals, we can only speculate whether this decreased arterial-venous oxygen content difference represented an increased cardiac output or a decreased oxygen consumption.

Albumin-exchanged animals at rest had significantly higher ($p < 0.05$) lactate levels compared to all other groups (Fig. 5). Lactate levels during exercise and recovery for the two albumin dogs capable of exercising following exchange were higher than levels found for either SFHS. However, the small sample size in this study limits our ability to compare these results on a statistical basis. Lactate levels for both hemoglobin solutions did not differ significantly from the Sham values for either rest, exercise, or recovery following exchange.

DISCUSSION

The ability of SFHS to maintain gross physiologic oxygen requirements following hemodilution has been well established (2,9,19). However, little has been done to investigate the effect of the relatively limited intravascular retention time of these solutions and their subsequent ability to act as an adequate resuscitative fluid beyond the initial exchange. Even less is known about the effects of introducing conditions such as exercise. The most significant work in the area of improving oxygen off-loading and vascular retention has been done by Greenburg and associates. They have evaluated the resuscitative efficiency of both modified and unmodified SFHS in dog and rat hypovolemic hypotensive shock models (10,12). These investigators have reported a half disappearance time of approximately 140 minutes for modified SFHS when mean systolic pressure was 70 TORR or greater. This value represents a 50% increase over similar results found with unmodified SFHS. They also reported that retention time varied inversely with pressure as renal function and the excretion of SFHS decreased with hypotension. From their results it would appear that modification of SFHS with pyridoxyl 5'-phosphate is a factor in increasing intravascular retention. However, their results were obtained in anesthetized animals studied shortly after resuscitation.

Immediate Post-Transfusion Period

All animals appeared well following exchange with any of the solutions, suggesting that none of the solutions were significantly toxic. However, the albumin-exchanged animals were clearly more limited in their ability to exercise, an activity requiring additional oxygen. Albumin-exchanged animals had significantly shorter total run times, higher resting heart rates, and higher resting venous lactate levels compared with either the hemoglobin group or the Sham group. Citing the apparent lack of oxyen as the reason for these

abnormalities is supported further by the significant decrease in arterial oxygen content in albumin-exchanged animals compared to the other groups.

It clearly appears that both hemoglobin solutions were able to transport enough oxygen to maintain exercise despite their differences in oxygen affinity. The more normalized P_{50} represented by modified SFHS did not provide any significant initial advantage over unmodified SFHS in terms of exercise ability; all results comparing these two solutions in the immediate post-transfusion period were indistinguishable. Furthermore, both solutions appeared capable of maintaining almost normal physiologic function since almost all of the values in the hemoglobin animals approached those achieved by the Sham dogs. Both hemoglobin groups experienced significant decreases in arterial oxygen content and this remains the one notable exception to the similarity in values among study groups. This appeared to be of little consequence in terms of exercise capacity because both hemoglobin groups exercised at levels only slightly lower than the Sham group.

These results are not totally unexpected. Much success has been reported in maintaining animals with SFHS even when exchanged to the zero hematocrit level. It now appears that SFHS, irrespective of increased oxygen hemoglobin affinity, appears capable of maintaining normal physiologic conditions in moderately hemodiluted dogs stressed with exercise.

The lower arterial-venous oxygen content difference values noted in the albumin-exchanged animals during exercise and recovery periods could be due to either increased cardiac output or decreased oxygen consumption. These animals were not instrumented in an appropriate manner for the measurement of cardiac output. However, in another animal study from our laboratories we instrumented pigs with vascular catheters and an aortic flow probe and subjected them to a period of treadmill exercise immediately following a 50%

exchange with albumin and unmodified SFHS (20). These animals also demonstrated a significantly decreased arterial-venous oxygen content difference which was accompanied by significantly decreased oxygen consumption and stable cardiac output. Furthermore, both animal models had an increase in lactate levels during exercise, indicating that the decreased oxygen consumption was not adequately meeting oxygen requirements. These results in two animal models would seem to support the thesis that the change in oxygen-carrying capacity between the albumin-exchanged and the SFHS animals is responsible for the observed physiologic differences.

Although there were no significant differences between the two hemoglobin solutions in the immediate post-transfusion period, by the 24-hour study time we did note some significant differentiation between the solutions. Arterial oxygen content, hematocrit, and heart rate levels remained virtually indistinguishable, however, the modified SFHS animals increased their exercise capacity at the 24-hour period, while unmodified SFHS animals experienced a 30% decrease. The mechanism for this difference is not apparent from the data presented here. Certainly it does not appear to be the result of increased intervascular retention or oxygen delivery potential, because arterial oxygen content levels between the two hemoglobin solutions were similar.

This beneficial effect may have its origin in other areas. It is possible to speculate that there may be a metabolic benefit associated with modified SFHS attributable to the increased molecules of glucose, adenine, and phosphate present in the modified solution rather than to any increased availability of oxygen. Since almost all of the solution has been excreted by 24 hours, it is difficult to conclude that the difference is due to improved oxygen off-loading. If the difference is indeed real, it must be attributed to some sequelae of a modified SFHS that persists beyond the presence of the solution itself.

Albumin-exchanged animals had an exercise response 24 hours post-transfusion that was limited but would show signs of recovery in terms of increased total run time, decreased heart rate, and slightly increased arterial oxygen content and hematocrit levels. It appears that normal metabolic and physiologic mechanisms were able to compensate for the limited oxygen availability resulting from the albumin transfusion.

Measurements made 48 hours post-transfusion show similar results for both albumin-exchanged and unmodified SFHS animals in all categories, suggesting that whatever benefit was initially provided by the unmodified hemoglobin solution was virtually absent at this time. This resulted primarily from the ability of the albumin-exchanged animals to raise their recovery values rather than from further deterioration of the SFHS animals. At the 48-hour testing, modified SFHS still appeared to maintain some advantage over the other exchange solutions since these animals still maintained an exercise capacity close to control, suggesting the continued presence of some form of beneficial metabolic activity.

By the seventh post-transfusion day all groups had returned to control levels in all categories except the albumin-exchanged animals. They continued to experience a significantly lower arterial oxygen content level. This decreased oxygen content appeared to correlate with the more persistently depressed hematocrit level still present in the albumin-exchanged animals by the seventh day. Perhaps the initial presence of SFHS may in some way accelerate the return of a more normal hematocrit as well as normal physiological functions, even though the SFHS is no longer in the vascular bed. Due to the unavailability of additional data in this area, these thoughts can only be regarded as speculative.

This study appears to provide support for several conclusions. Moderate hemodilution with a more normalized P_{50} does not provide any initial advantage in terms of exercise capacity. However, the modified SFHS did appear to provide some advantage over unmodified SFHS in terms of exercise capacity at the 24- and 48-hour post-transfusion periods. Both hemoglobin solutions provide a significant advantage over 7% albumin solution as a resuscitative fluid. However, these advantages appear to be short-lived and are of little benefit 48 hours post-transfusion.

By the seventh post-transfusion day, all animal groups approached control levels and were virtually indistinguishable, although there is some evidence that the SFHS groups may have obtained this control level at a slightly faster rate. The initial beneficial effects appear to be due to the increased oxygen-carrying capacity of the hemoglobin solutions and we speculate that the more subtle long-range benefits arise from an effect that outlasts the actual presence of the solutions.

A final conclusion relates to our failure to document any signifiant non-stroma related toxicity such as that reported by others. The recent documentation of toxicity by other investigators who have taken great care to evaluate many forms of hemoglobin solutions continues to be disturbing. When this information is combined with similar toxic reactions noted in humans (21), then continued caution must be exercised in using these solutions in clinical applications.

In the search for an ideal "blood substitute," even our imperfectly developed stroma-free hemoglobin solution with limited oxygen off-loading and intervascular retention should be considered a leading contender.

REFERENCES

1. Bonhard K. Acute oxygen supply by infusion and hemoglobin solutions. Fed Proc 1975; 34:1466-467.

2. Moss GS, DeWoskin R, Rosen AL, Levine H, Palani CK. Transport of oxygen and carbon dioxide by hemoglobin saline solution in the red cell free primate. Surg Gynecol Obstet 1978; 142:357-362.

3. Devenuto F, Friedman HI, Neville JR, Peck CC. Appraisal of hemoglobin solutions as blood substitutes. Surg Gynecol Obstet 1979; 149:417-436.

4. Sasuzuhn T. Studies of neptaglobin. I. Immunochemic properties of heptaglobin and antihemoglobin antibody. Proc Jap Acad 1970; 46:820- .

5. Cochin A, Das Gupta TK, Dwoskin R, Moss GS. Immunogenic properties of stroma vs. stroma-free hemoglobin solution. Surg Forum 1972; 23:19-21.

6. Rabiner SF. Evaluation of a stroma-free hemoglobin solution for use as a plasma expander. J Exp Med 1967; 126:1127-1142.

7. Rabiner SF. Hemoglobin solution as a plasma expander. Fed Proc 1975; 34(6):1454-1457.

8. Usami S, Chien S, Gregerson MI. Hemoglobin solution as a plasma expander: Effects of blood viscosity. Proc Soc Exp Biol Med 1971; 136:1232-1235.

9. Moores WY, DeVenuto F, Heydorn WH, et al. Extending the limit of hemodilution on cardiopulmonary bypass using stroma-free hemoglobin solution. J Thorac Cardiovasc Surg 1981; 81:155-162.

10. Greenburg AG, Elia C, Levine B, Belsha J, Peskin GW. Hemoglobin and the oxyhemoglobin dissociation curve. J Trauma 1975; 5:943-950.

11. Greenburg AG, Peskin GW, Hoyt DB, Moores WY. Is it necessary to improve the intravascular retention of hemoglobin solutions? Crit Care Med 1982; 10:266-269.

12. Greenburg AG, Schooley M, Peskin GW. Improved retention of stroma free hemoglobin solution by chemical modification. J Trauma 1977; 17:501-504.

13. Peskin GW, O'Brien K, Kabiner SF. Stroma free hemoglobin solution: The "ideal" blood substitute? Surgery 1969; 66:185-193.

14. Kabiner SF, O'Brien K, Peskin SB, Friedman LH. Further studies with stroma free hemoglobin solution. Ann Surg 1970; 171:615-622.

15. White CT, Murray AJ, Smith DJ, Greene JR, Bolin RB. Synergistic toxicity of endotoxin and hemoglobin. J Lab Clin Med, 1986; 108:132-137.

16. White CT, Murray AJ, Greene JR, et al. Toxicity of human hemoglobin solution infused into rabbits. J Lab Clin Med, 1986; 108:121-131.

17. Kothe K, Eichentopf B, Bonhard K. Characterization of a modified stroma-free hemoglobin solution as an oxygen-carrying plasma substitute. Surg Gynecol Obstet 1985; 161:563-569.

18. Beutler E. Red Cell Metabolism: A Manual of Biochemical Methods. New York: Grune & Stratton, 1971.

19. DeVenuto F, Moores WY, Zegna AI, Zuck TF. Total and partial blood exchange in the rat with hemoglobin prepared by crystallization. Transfusion 1977; 17:655-662.

20. Mack RE, Moores WY, White FC, et al. Improved exercise performance in hemodiluted pigs with stroma-free hemoglobin. Manuscript submitted to J Appl Physiol.

21. Savitsky JP, Doczi J, Black J, Arnold JD. A clinical safety trial of stroma-free hemoglobin. Clin Pharmacol Ther 1978; 23:73.

Figure Legends

Figure 1 Total run times prior to exchange (control), immediately following exchange, 24 hours, 48 hours, and seven days following exchange. *p<0.05 (albumin exchange vs. others), + p<0.05 exchange vs. control. Values as mean ± S.D.

Figure 2 Hematocrit values prior to exchange (control), immediately following exchange, 24 hours, 48 hours, and seven days following exchange. *p<0.05 (albumin exchange vs. others), + p<0.05 exchange vs. control. Values as mean ± S.D.

Figure 3 Heart rates before and immediately following exchange. *p<0.05 (albumin exchange vs. others), + p<0.05 exchange vs. control. A. Resting heart rates. B. Maximum exercise heart rates. C. Recovery heart rates. Values as mean ± S.D.

Figure 4 Arterial oxygen content prior to exchange (control), immediately following exchange, 24 hours, 48 hours, and seven days following exchange. *p<0.05 (albumin exchange vs. others), + p<0.05 exchange vs. control. Values as mean ± S.D.

Figure 5 Histogram representing venus lactate values at rest before and after exchange transfusions. *p<0.05 albumin exchange vs. others. SHAM-sham transfusion, ALBUMIN-albumin transfusion, U-SFHS unmodified stroma-free hemoglobin solution, M-SFHS-modified stroma free hemoglobin solution. Values as mean ± S.D.

TABLE 1

ARTERIAL-VENOUS OXYGEN CONTENT DIFFERENCE

ARTERIAL-VENOUS OXYGEN CONTENT DIFFERENCE ($cc/O_2/dl$)	SHAM		ALBUMIN		UNMODIFIED STROMA-FREE HEMOGLOBIN		MODIFIED STROMA-FREE HEMOGLOBIN	
	CONTROL	EXCHANGE	CONTROL	EXCHANGE	CONTROL	EXCHANGE	CONTROL	EXCHANGE
1. Rest	4.9 ± 0.9	4.5 ± 1.4	5.8 ± 1.3	3.4 ± 1.5	4.7 ± 2.1	3.8 ± 1.8	6.2 ± 1.3	5.3 ± 0.6
2. Exercise	8.3 ± 0.8	7.8 ± 2.2	8.2 ± 1.8	4.5 ± 1.2*	9.3 ± 2.4	6.5 ± 1.4	9.3 ± 2.1	6.9 ± 0.5
3. Recovery	4.8 ± 0.8	6.3 ± 0.4	5.3 ± 2.0	3.6 ± 0.4*	4.7 ± 1.5	4.5 ± 1.0	6.9 ± 0.7	5.2 ± 0.5

Control and exchange results for animals transfused with either their own blood (Sham), 7% albumin, unmodified stroma-free hemoglobin solution, or modified stroma-free hemoglobin solution.

* n=2, as only 2 of 5 albumin-exchanged animals were capable of exercising post-transfusion.

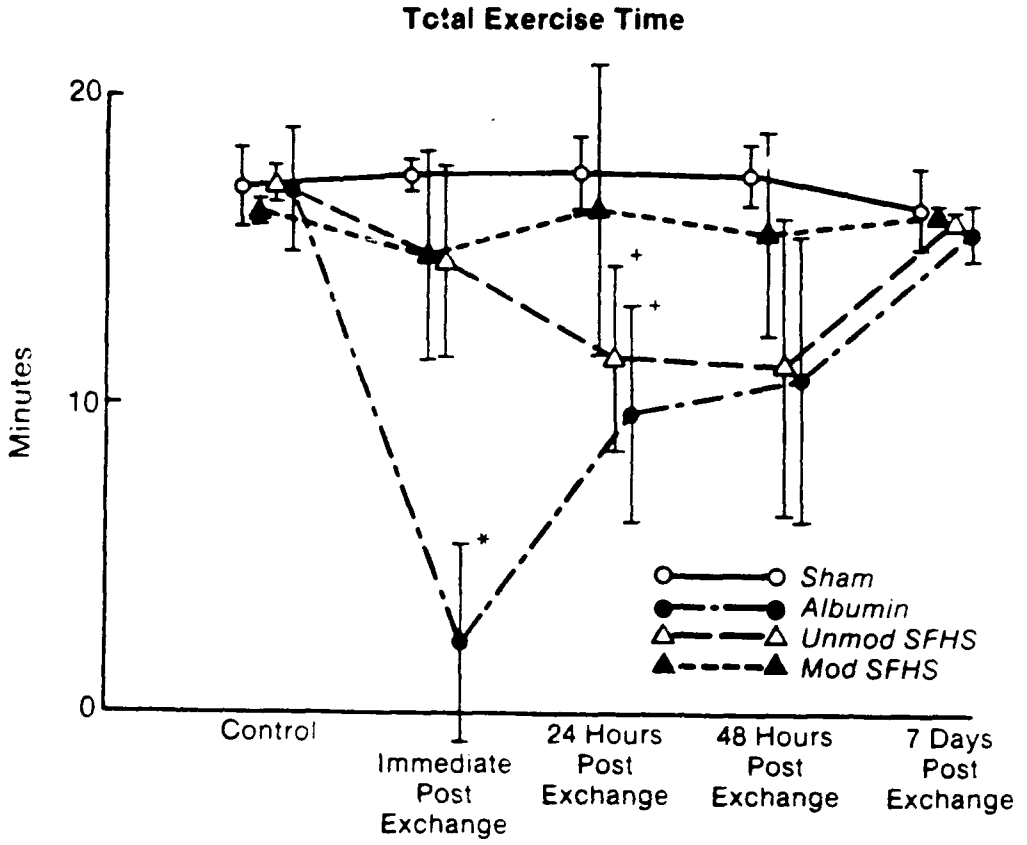
Total Exercise Time
20
10
0
Minutes
Control
Immediate Post Exchange
24 Hours Post Exchange
48 Hours Post Exchange
7 Days Post Exchange
Sham
Albumin
Unmod SFHS
Mod SFHS
*
+
+

FIGURE 1

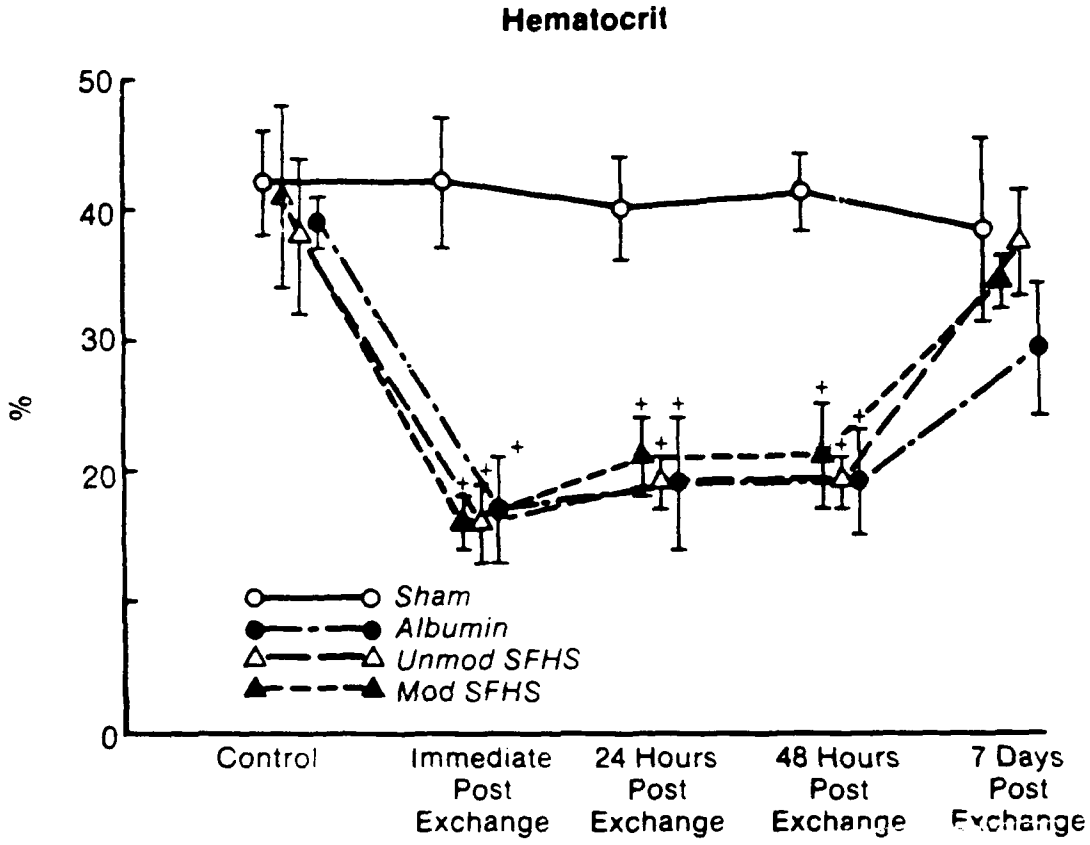
Hematocrit
50
40
30
20
0
%
Sham
Albumin
Unmod SFHS
Mod SFHS
Control
Immediate Post Exchange
24 Hours Post Exchange
48 Hours Post Exchange
7 Days Post Exchange

FIGURE 2

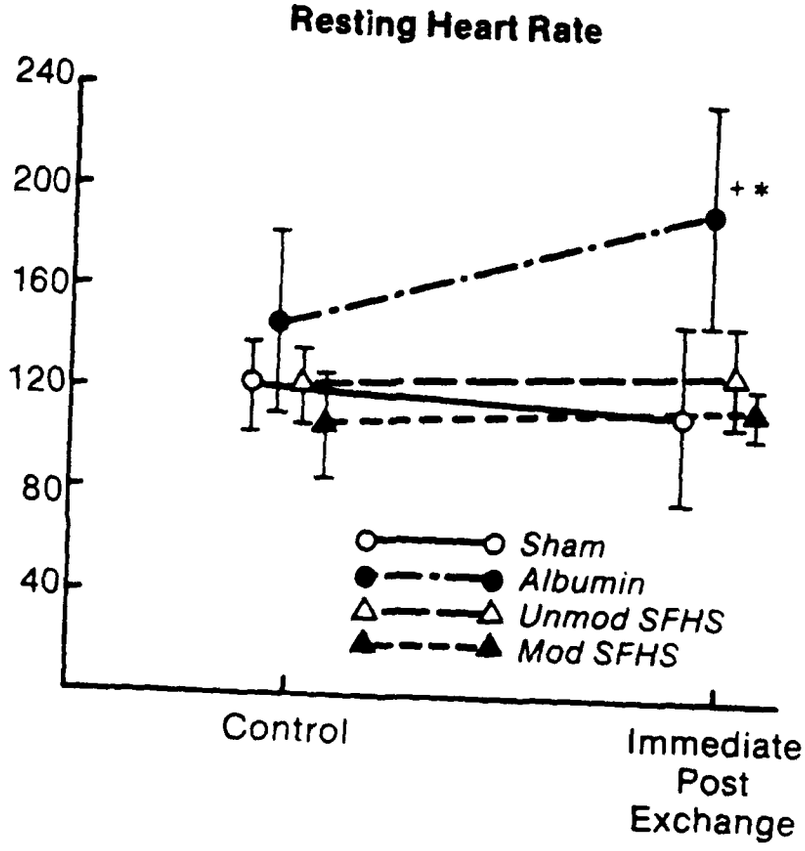
Resting Heart Rate
240
200
160
120
80
40
+ *
Sham
Albumin
Unmod SFHS
Mod SFHS
Control
Immediate
Post
Exchange

FIGURE 3A

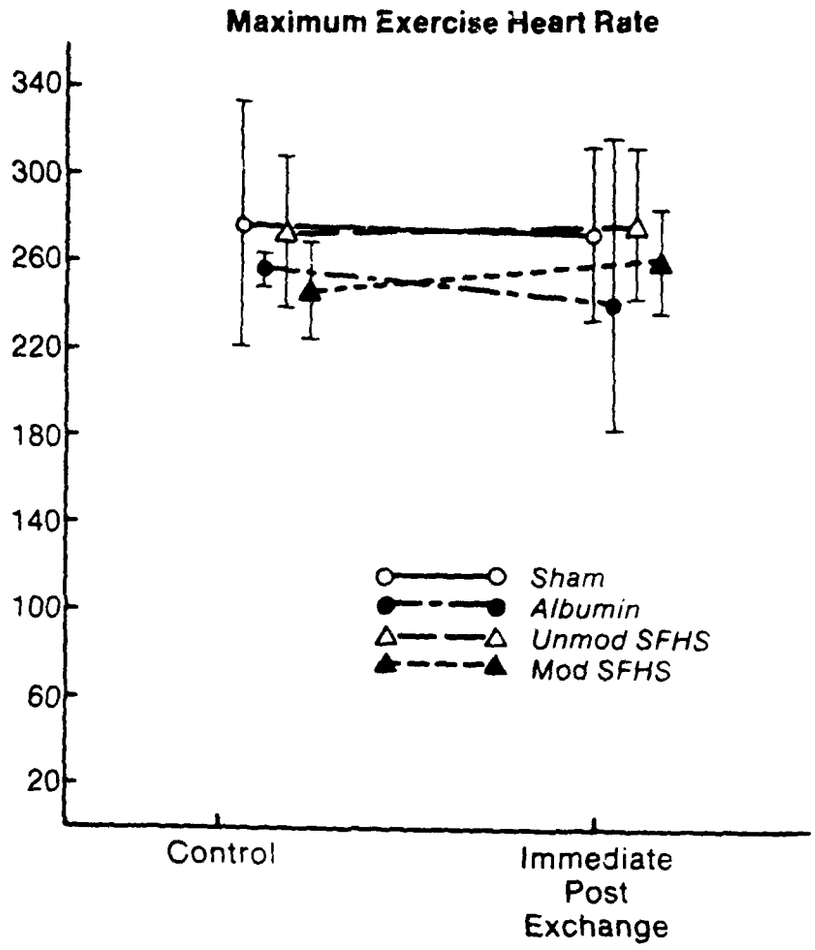
Maximum Exercise Heart Rate
340
300
260
220
180
140
100
60
20
Sham
Albumin
Unmod SFHS
Mod SFHS
Control
Immediate
Post
Exchange

FIGURE 3B

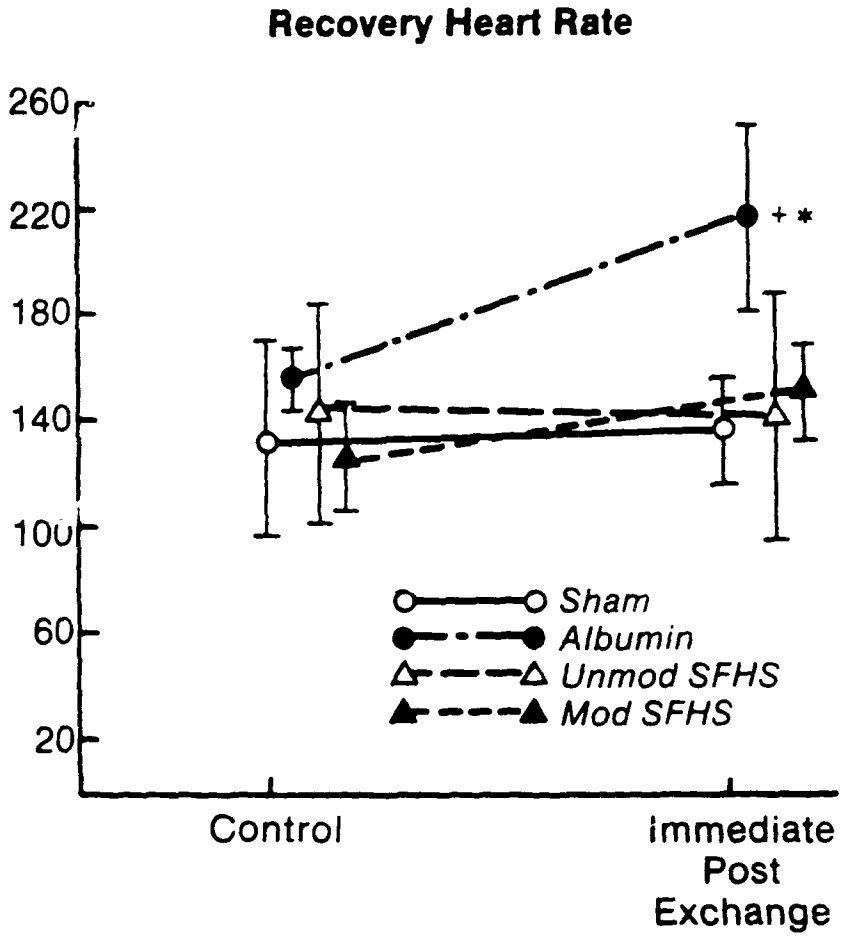
Recovery Heart Rate
260
220
180
140
100
60
20
+ *
Sham
Albumin
Unmod SFHS
Mod SFHS
Control
Immediate Post Exchange

FIGURE 3C

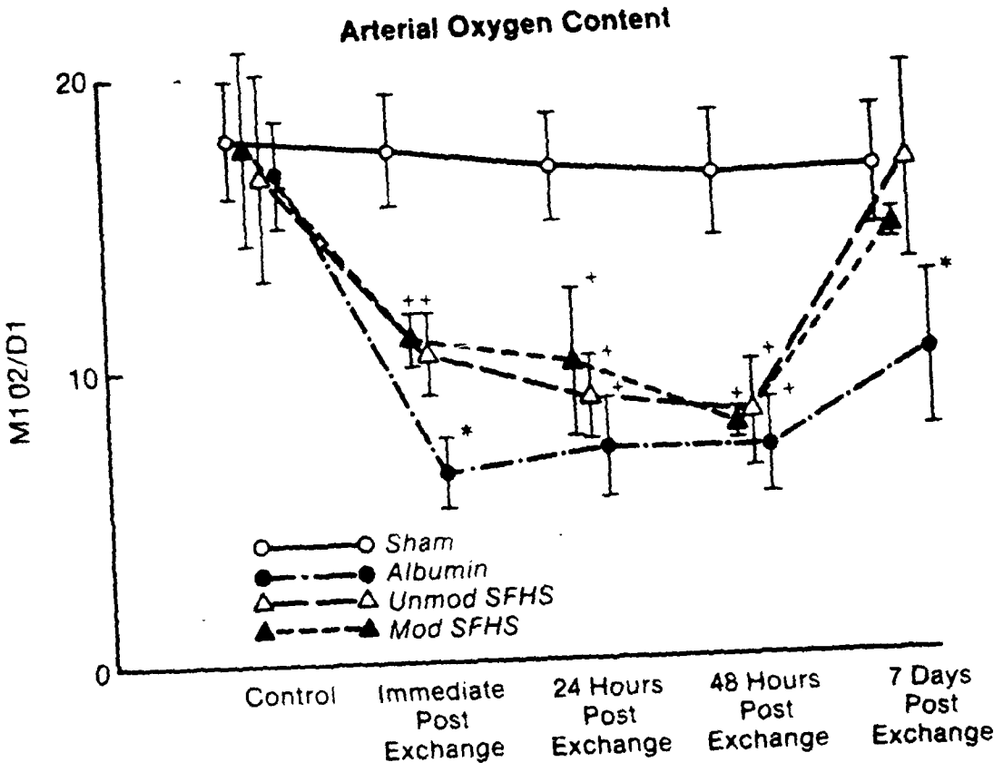
Arterial Oxygen Content
20
10
0
M1 02/D1
Sham
Albumin
Unmod SFHS
Mod SFHS
Control
Immediate Post Exchange
24 Hours Post Exchange
48 Hours Post Exchange
7 Days Post Exchange

FIGURE 4

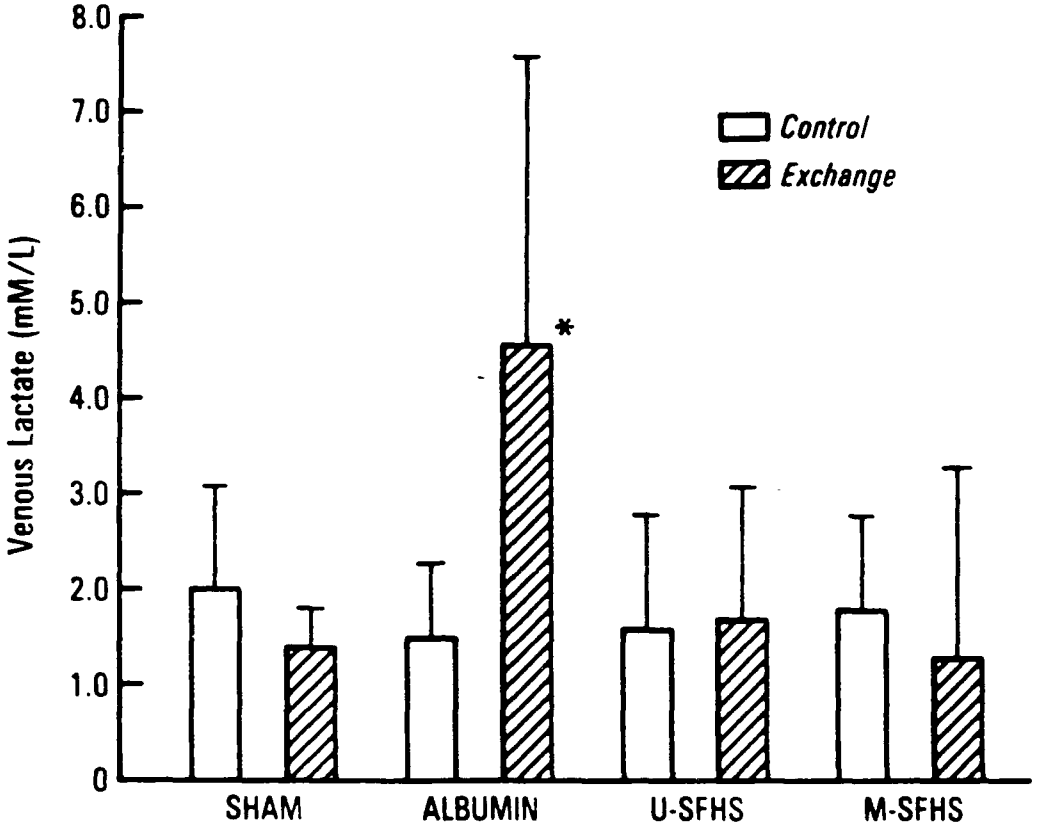
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0
Venous Lactate (mM/L)
Control
Exchange
*
SHAM
ALBUMIN
U-SFHS
M-SFHS

FIGURE 5

(6)

CORONARY FLOW DYNAMICS IN SWINE FOLLOWING PARTIAL EXCHANGE TRANSFUSIONS WITH HEMOGLOBIN AND ALBUMIN SOLUTIONS

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Stroma-free hemoglobin solution (SFHS) has been found to be an effective blood substitute by many investigators who have shown its efficacy in total and partial exchange models. Unfortunately, other reports have documented significant toxicity (bradycardia and increase blood pressure) in both animal studies (1) and human studies (2). We evaluated this problem utilizing a pig chronically instrumented with an electromagnetic flow probe and pressure and sampling catheters. This model allowed us to make detailed coronary flow dynamic measurements in an unanesthetized animal subjected to a treadmill exercise stress.

Methods: Twelve swine (40-50 kg) of either sex were chronically instrumented in a manner previously reported (3). Left ventricular internal diameter ultrasonic dimension crystals were implanted as indicators of global heart mechanics. Silastic catheters were placed in the descending thoracic aorta, the pulmonary artery, and the left atrium. An electromagnetic flow probe was placed around the ascending aorta to measure output. All catheters and lead wires were brought out of the thoracic cavity via the fourth intercostal space and then run subdermally to the back, where they were externalized. Distribution of cardiac output was determined by injection of carbonized microspheres (15±10μm) and regional blood flow was calculated allowing organ flows to be expressed in ml/min/g tissue. Four conditions were studied in each animal: 1) control rest; 2) control exercise; 3) exchange rest, and 4) exchange exercise. The following measurements were obtained at each condition: 1) cardiac output; 2) arterial and left atrial pressure; 3) sonomicrometry-measured ventricular dimensions; and 4) microsphere-determined organ flow. The two solutions prepared for these experiments included: 1) a 7% albumin solution prepared using serum bovine albumin from a commercial source, and 2) a 7% SFHS prepared using a modification of the technique described by Greenburg.

Results: The results are summarized in Table I. Exercise generally resulted in an increased cardiac output and aortic pressure; the greatest blood pressure was with the combination of SFHS and exercise ($p<0.05$ SFHS vs. control and albumin exercise). The exercise blood pressure response was blunted with albumin, which

TABLE I

	Albumin Solution				Hemoglobin Solution			
	Control		Test		Control		Test	
	Rest	Exer.	Rest	Exer.	Rest	Exer.	Rest	Exer.
Aortic Blood Pressure (mmHg)	114± 9	131± 12	97± 19	107± 12†	105± 15	127± 13	123± 15	150± 22*
Cardiac Output (ml/min/kg)	116± 31	260± 86	179± 45 x	264± 57	98± 19	211± 36	99± 13	251± 37
Coron. Resist. (mmHg/ml/100g)	1.11± 0.15	0.39± 0.09	0.38± 0.98	0.15± 0.04	0.93± 0.19	0.29± 0.08	0.58± 0.18	0.24± 0.03
Coronary Flow (ml/min/100)	100± 29	318± 79	310± 167 x	652± 182†	106± 30	424± 139	178± 59	568± 109
Diameter Shortening (%)	26± 11	27± 12	27± 10	25± 9	29± 6	32± 9	25± 1	35± 7

†p<0.05 vs. control; *p<0.05 vs. control, albumin solution; xp<0.05 vs.control, SFHS

had a significantly lower pressure (p<0.025) with exercise following exchange with albumin. Coronary blood flow changes occurred with exercise and exchange, but the changes over control values (both at rest and with exercise) reached statistical significance (p<0.025) only with the albumin exchange. When these coronary flow results were combined with the pressure drops across the myocardium to obtain coronary resistance values there were significant reductions with both exchange and exercise, however when the resistance changes from the control conditions were compared between the albumin and SFHS exchanged animals there were no significant differences. In neither case did the exchange transfusion result in an increased coronary resistance, and the measured decrease in coronary resistance was somewhat less with SFHS than with albumin. Systolic performance, as measured by diameter shortening, did not differ with exchange using either solution.

Discussion: The results in these animals provides evidence for both the efficacy and safety of SFHS, although there were physiologic changes from the control situation with exchange transfusions using either solution. However, these changes were generally less with SFHS.. There was no evidence for coronary vasoconstriction with SFHS and the vasodilitation response was consistent with the resultant drop in oxygen content that occurred with either SFHS or albumin solution exchange.

REFERENCES

1. C.T. White, A.J. Murray, J.R. Greene, D.J. Smith, F. Medina, G.T. Makovec, E.J. Martin, R.B. Bolin, J. Lab. Clin. Med. (in press 1986)
2. J.P. Savitsky, J. Doczi, J. Black, J.D. Arnold, Clin. Pharmacol. Ther., 23, 73 (1978)
3. M. Sanders, F.C. White, C.M. Bloor, Comp. Biochem. Physiol., 58, 365-370 (1979)